The American Journal of Medicine

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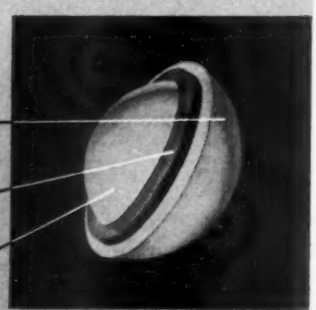

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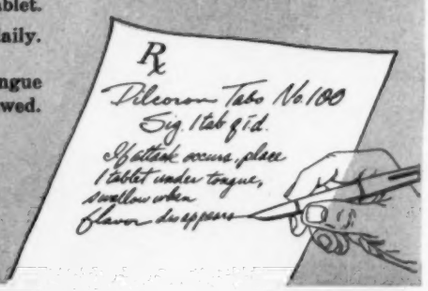

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The American Journal of Medicine

Vol. XXIV JANUARY, 1958 No. 1

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Clinical Studies

Characterization of the Anemia Associated with Chronic Renal Insufficiency
J. PHILIP LOGE, ROBERT D. LANGE AND CARL V. MOORE 4

This careful study of the anemia occurring in twenty-six patients with chronic renal insufficiency of various etiology confirms previous findings indicating that depression of erythropoiesis, here established convincingly by demonstration of poor utilization of radioiron, is the principal immediate cause. In some cases it was established that an unidentified extracorpuscular hemolytic factor (or factors) also operates to accelerate the rate and intensity of red blood cell disappearance. Hemorrhage may be a third, compounding cause of anemia in patients with chronic renal insufficiency.

Abnormal Hemoglobins in the Negroes of Surinam
CLAIRE LIACHOWITZ, JANET ELDERKIN, IWAN GUICHIRIT, HAROLD W. BROWN AND HELEN M. RANNEY 19

There has been much interest in the indications that the sickle cell trait offers some protection against Plasmodium falciparum infection among African natives living in hyperendemic malarial areas. The present study examines this question in the Djukas of Surinam, also heavily infested with P. falciparum. The incidence of P. falciparum parasitemia was found to be about the same in "sicklers" and in "non-sicklers." Some interesting collateral observations were made in the course of study.

Fever, Infection and Host Resistance in Acute Leukemia
RICHARD T. SILVER, JOHN P. UTZ, EMIL FREI, III AND NORMAN B. MCCULLOUGH 25

Fever is common in patients with acute leukemia, and ordinarily one casually decides whether the fever is due to the underlying disease or to some secondary infection by desultory cultures and by the response to antibiotics. The present authors were not content with this disposition of the matter and made an exhaustive study of bacterial, fungal and viral infections in the causation of fever in thirty-six patients with acute leukemia. They were able to demonstrate infectious organisms or infected sites in about two-thirds of the febrile episodes in these patients. The details, and the implications for management, are set forth at length.

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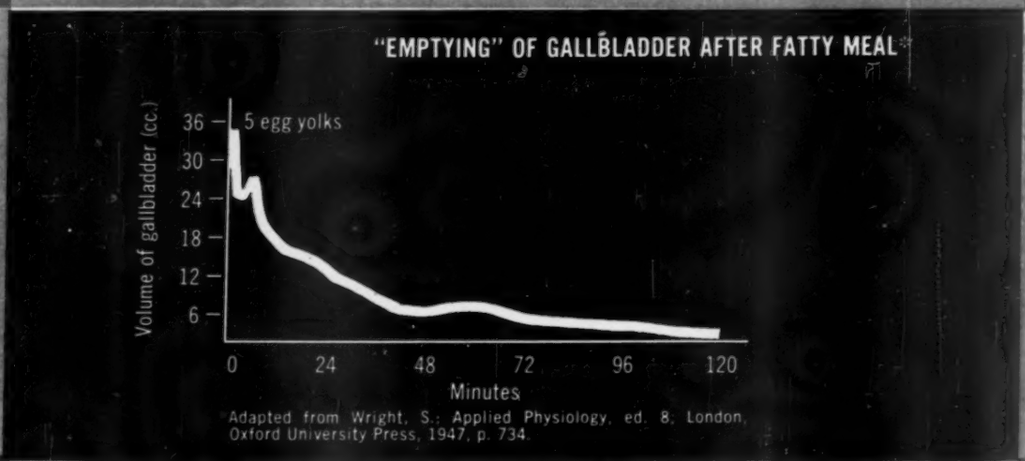
AN AMES CLINIQUICK

CLINICAL BRIEFS FOR MODERN PRACTICE



What's wrong with the term "emptying of the gallbladder"?

The gallbladder discharges bile by fractional evacuation. It is not emptied completely at any one time even following a fatty meal.

Source—Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bile Ducts, ed. 3, Philadelphia, Lea & Febiger, 1953, vol. 2, p. 1177.

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VOLUME TWENTY-FOUR NUMBER ONE

Methods for Analyzing Survival Data, Illustrated by Hodgkin's Disease
EDWIN E. OSGOOD 40

Meaningful evaluation of therapy, for example of the effectiveness of radiation or chemotherapy for prolongation of survival in the neoplastic diseases, requires proper experimental design, procurement of the appropriate data and valid statistical analysis. Dr. Osgood expands upon some of these points in connection with current modes of treatment of Hodgkin's disease. The use of reference logarithmic probability curves and of the random number sign test is illustrated. He finds, upon analysis of the available data, that the introduction of antibiotics and alkylating agents has effected no significant improvement in the survival time of patients with Hodgkin's disease.

Cytomegalic Inclusion Disease in Adults. A Complication of Neoplastic Disease of the Hemopoietic and Reticulohistiocytic Systems . . . ROBERT JOSEPH PEACE 48

This interesting report calls attention to an uncommon but doubtless frequently overlooked or misinterpreted complication, in the adult, of debilitating disease—dissemination of salivary gland virus. Such dissemination is most marked in the lungs and adrenals, where typical lesions characterized by cytomegalic inclusions are found. Three illustrative cases are cited.

Short-term Penicillin and Dihydrostreptomycin Therapy of Streptococcal Endocarditis. Results of the Treatment of Thirty-five Patients
RALPH TOMPSETT, WILLIAM C. ROBBINS AND CARL BERNTSEN, JR. 57

Of thirty-five patients with streptococcal endocarditis treated for fourteen days with combined penicillin and dihydrostreptomycin therapy, definite or probable bacteriologic cure was achieved in 91 per cent, thus supporting the general acceptance of this combination of antibiotics as offering the most satisfactory basic regimen for the management of patients harboring streptococcal strains which are sensitive to penicillin in *in vitro* tests. However, the occurrence of bacteriologic relapse in 10 per cent of cases properly raised the question of adequate duration (fourteen days) of antibiotic therapy. The authors recommend routine extension of drug administration for an additional seven to fourteen days. Valid theoretical as well as statistical reasons are adduced to support this advice.

ACTH and Adrenal Steroids in the Treatment of Pneumococcal Meningitis in Adults
JOHN C. RIBBLE AND ABRAHAM I. BRAUDE 68

The authors emphasize the hazards of vigorous exudative and hypersensitivity reactions in pneumococcal meningitis and point out that the use of corticotropin, as a supplement to bactericidal chemotherapy, would be rational in reducing these reactions. Acting upon this idea, twelve adults with pneumococcal meningitis were treated with the combined therapeutic regimen. The results were conspicuously favorable inasmuch as eleven survived, and without residua. This form of management would seem to deserve further trial.

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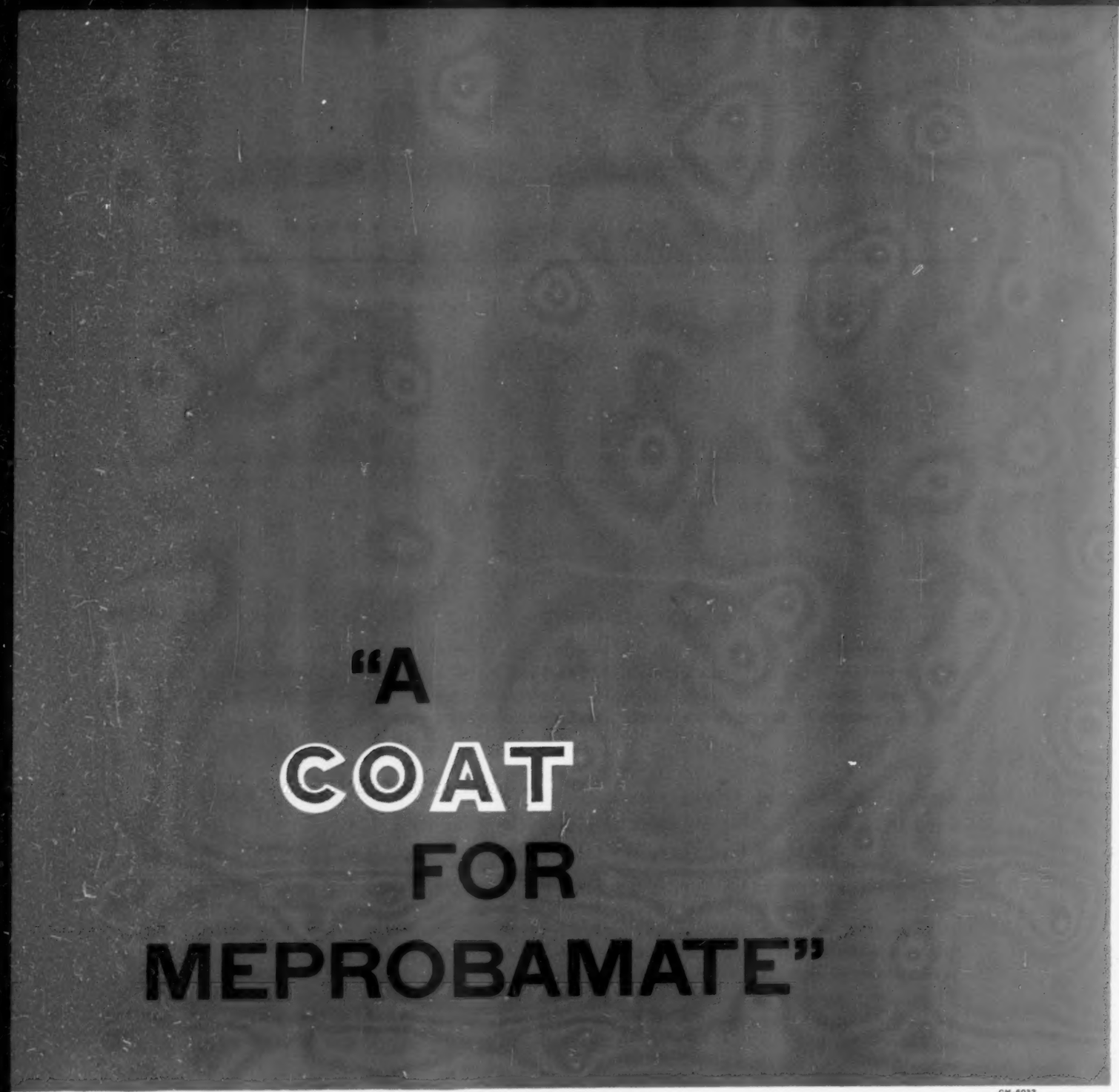

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The Influence of Androgens, Estrogens and Related Steroids on Serum Lipids and Lipoproteins. Observations in Hypogonadal and Normal Human Subjects
ROBERT H. FURMAN, R. PALMER HOWARD, LEONARD N. NORCIA AND E. CORINNE KEATY 80

The sex differences in vulnerability to coronary atherosclerosis have focused attention upon the effects of gonadal steroids on the serum lipids and lipoproteins, both from the point of view of the pathogenesis of atherosclerosis and its possible prevention. The present investigation is concerned chiefly with ultracentrifugation (flotation) studies for the most part in hypogonadal subjects presumably relatively free of atherosclerosis. A variety of potent and weak estrogenic and androgenic compounds were tested. The most consistent finding was a prompt increase in high density (alpha) lipoproteins after administration of estrogens, and a prompt decrease in this fraction caused by androgens; the effects on low density (beta) lipoproteins were less impressive but generally in the reverse direction. Corresponding changes were observed in serum cholesterol and phospholipid concentrations. The implications of these findings are discussed with reasonable caution and restraint.

Review

Right-sided Bacterial Endocarditis and Endarteritis. A Clinical and Pathologic Study
ROBERT C. BAIN, JESSE E. EDWARDS, CHARLES H. SCHEIFLEY AND JOSEPH E. GERACI 98

This timely study brings the classic reviews of right-sided bacterial endocarditis up to date by emphasizing the changes wrought since the introduction of antibiotics. The authors begin with an enlightening consideration of the factors which encourage right-sided rather than the much more common left-side implantation. They then review the clinical, laboratory and autopsy findings in their series of twenty-three cases. A number of interesting points are brought out: the increasing prevalence of Micrococcus pyogenes as the causative organism in right-sided endocardial lesions, the frequent absence of audible murmurs in tricuspid endocarditis, the high incidence of fatal septic pulmonary infarction, the nature of associated myocardial and renal lesions.

Seminar on Liver Disease

Bilirubin Metabolism in Jaundice . . . BARBARA H. BILLING AND G. H. LATHE 111

The symposium on liver disease is appropriately introduced by this authoritative account of recent investigation in the field of bilirubin metabolism and its implications in respect to the pathogenesis of jaundice. The authors begin with a review of the work leading to identification of the glucuronic acid conjugates of bilirubin and of the enzyme systems involved in their biosynthesis. They then consider the effect of biliary tract obstruction, hepatitis and hemolytic disease on plasma levels of bilirubin, pigment I and pigment II. The final sections deal with jaundice in the newborn, congenital hyperbilirubinemia and kernicterus. One completes study of this article with new insight into the diseases causing jaundice in man.

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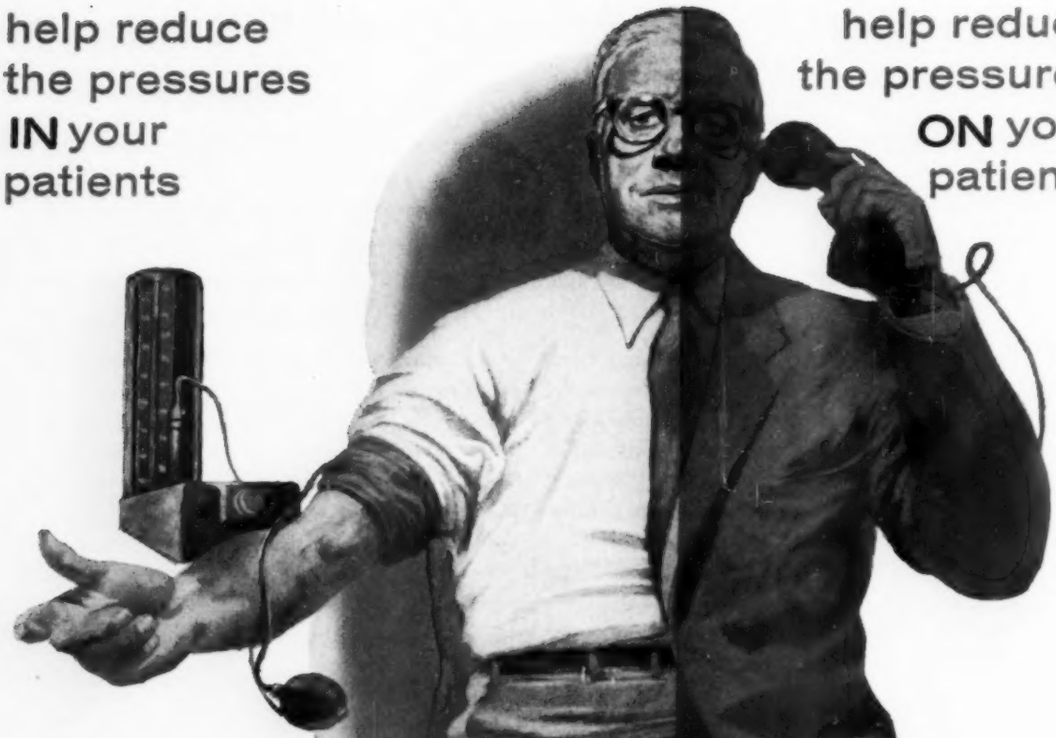

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Corrin, K. M.: Am. Pract. & Dig. Treatment 8:721 (May) 1957.

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Polycythemia 132

Combined Staff Clinic (Columbia University College of Physicians & Surgeons)—The Clinic begins by defining the term "polycythemia" and the two main forms of polycythemia, primary and secondary. The latter is first considered, with special reference to the hypoxemic polycythemia occurring with protracted exposure to reduced oxygen tensions, e.g., high altitude, and the polycythemia associated with excessive methemoglobinemia. Polycythemia vera is next discussed, first in relation to the occasional patient with significant unsaturation of arterial blood oxyhemoglobin (ordinarily a characteristic of secondary polycythemia), then from the point of view of erythropoiesis and iron metabolism. The last section is a sober appraisal of the current status of the question of a specific hormonal factor which regulates red blood cell production.

Case Reports

Chronic Idiopathic Immunoneutropenia . . . . . JOHN J. BUTLER 145

An interesting example of a phenomenon which may well be more widely distributed than is appreciated.

Acute Renal Failure Responding to Potassium Replacement Therapy
ARTHUR L. GROPPER 153

An instructive case.

Fulminating Uremic Pneumonitis Associated with Acute Ischemic Nephropathy (Lower Nephron Nephrosis)
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An interesting case.

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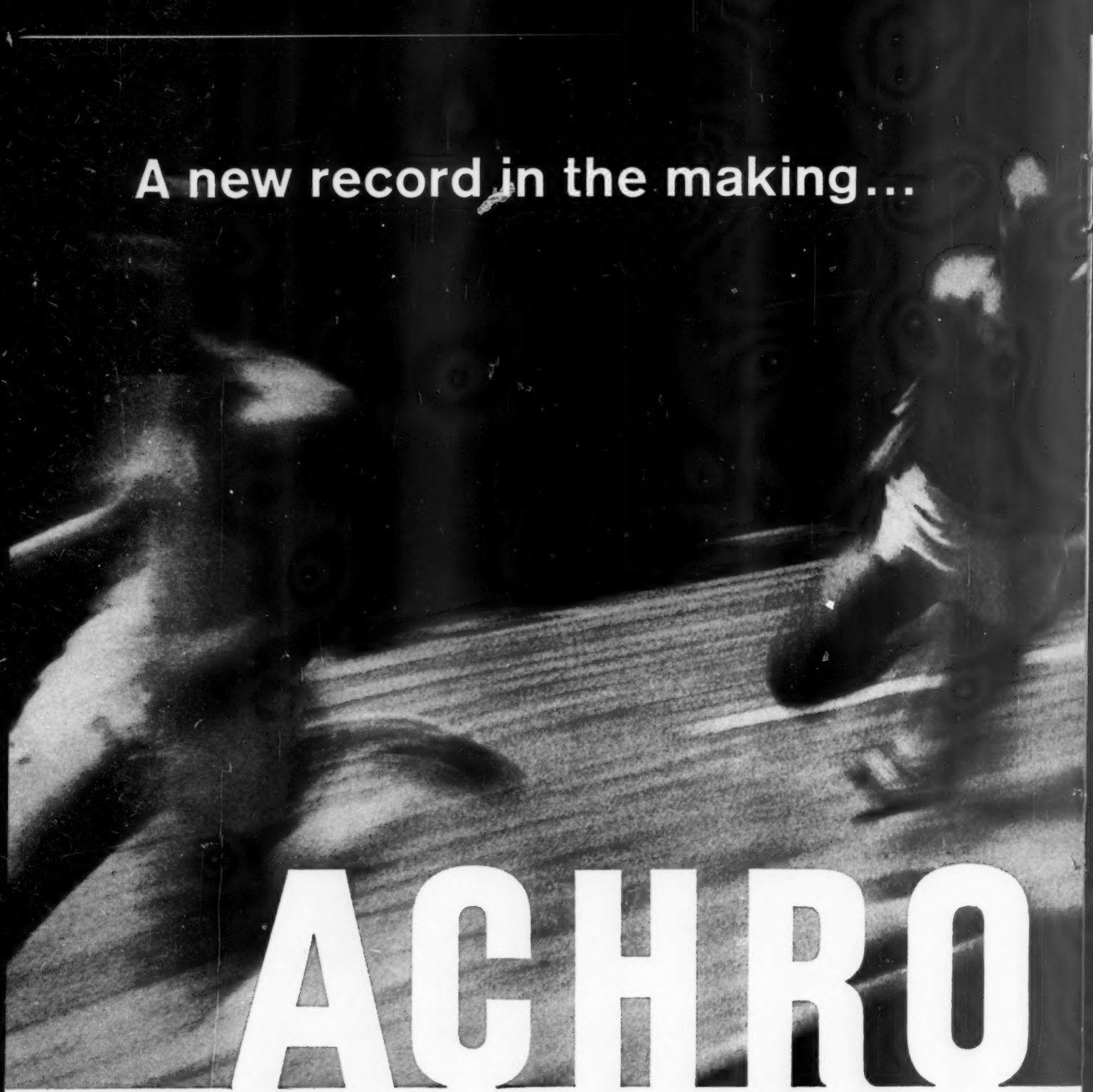

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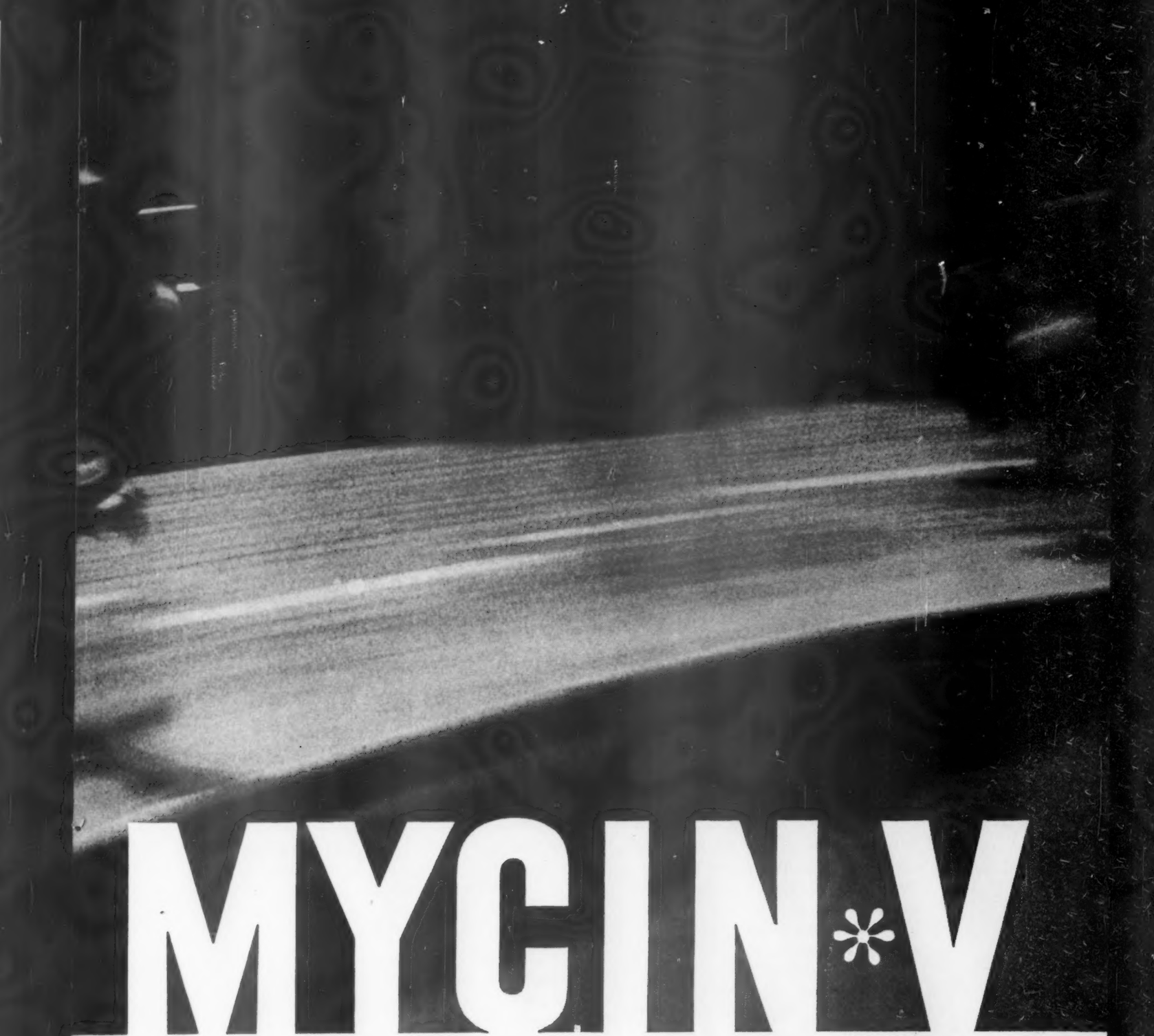

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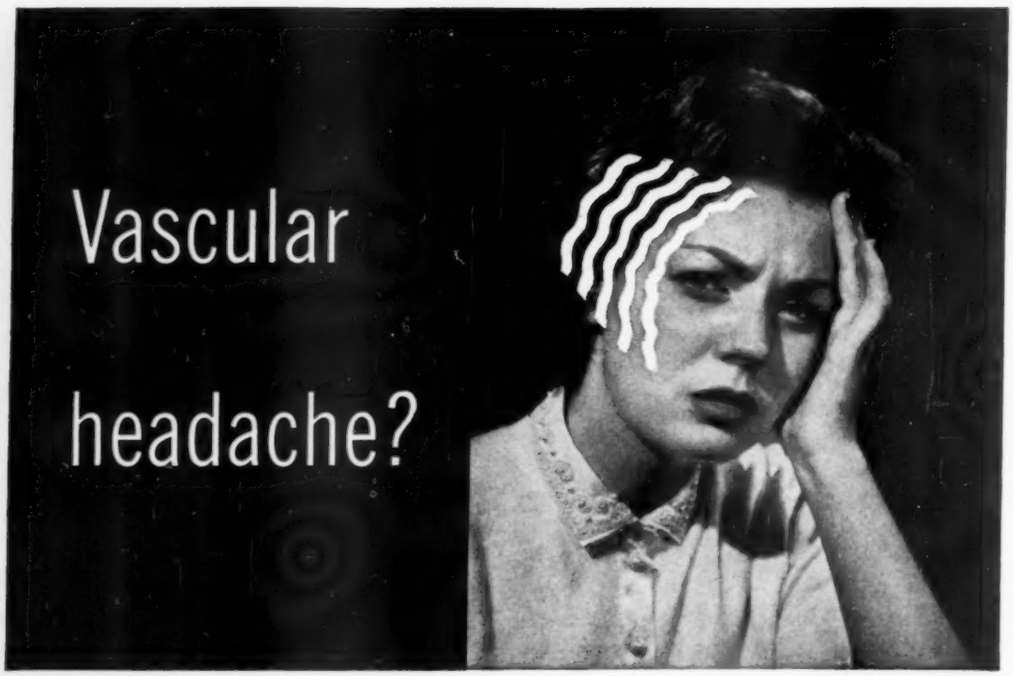

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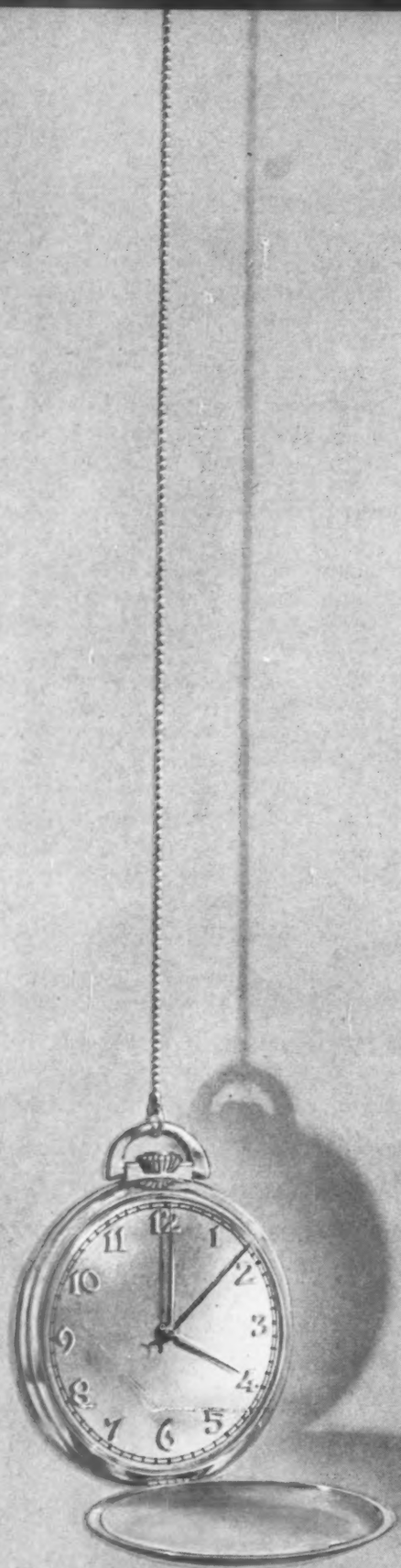

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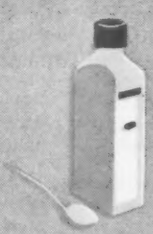

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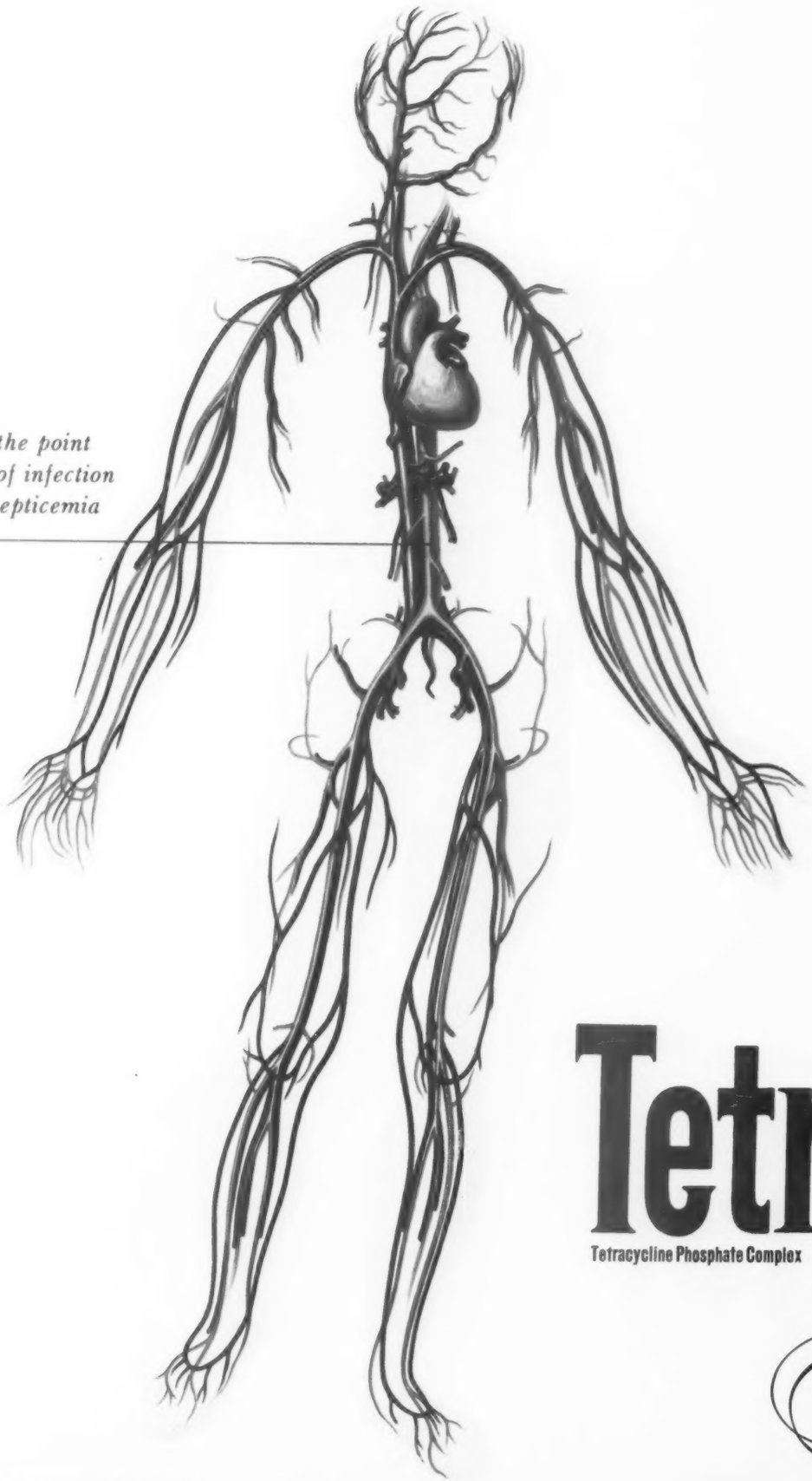

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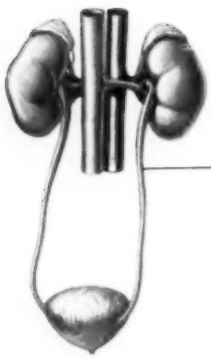

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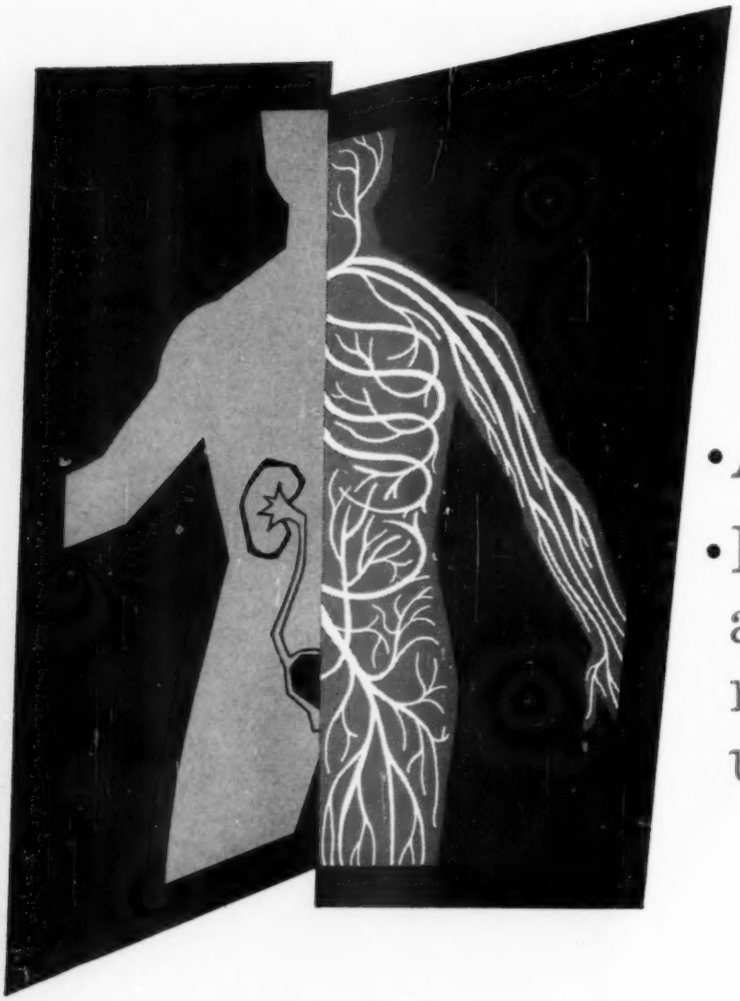
intensified tetracycline control with TETREX */ potentiated in anti-infective concentration and range* by sulfamethizole — outstanding sulfonamide for solubility, absorption, safety */ plus the remarkably rapid and specific g.u. analgesic action* of phenylazo-diamino-pyridine HCl in dysuria, frequency and urgency

Each AZOTREX Capsule contains: TETREX (tetracycline phosphate complex) 125 mg. (tetracycline HCl activity)
Sulfamethizole . . . . . 250 mg.
Phenylazo-diamino-pyridine HCl . . . 50 mg.
Minimum adult dose: One capsule q.i.d.
Supplied: Bottles of 24 and 100 capsules.



- Angiography
- Intravenous and retrograde urography

Versatile radiological agents with an unexcelled degree of safety

RENOGRAFIN

Squibb Contrast Media

- **well tolerated systemically and locally**
- **consistently good visualization**
- **convenient concentrations, in easily administered form**

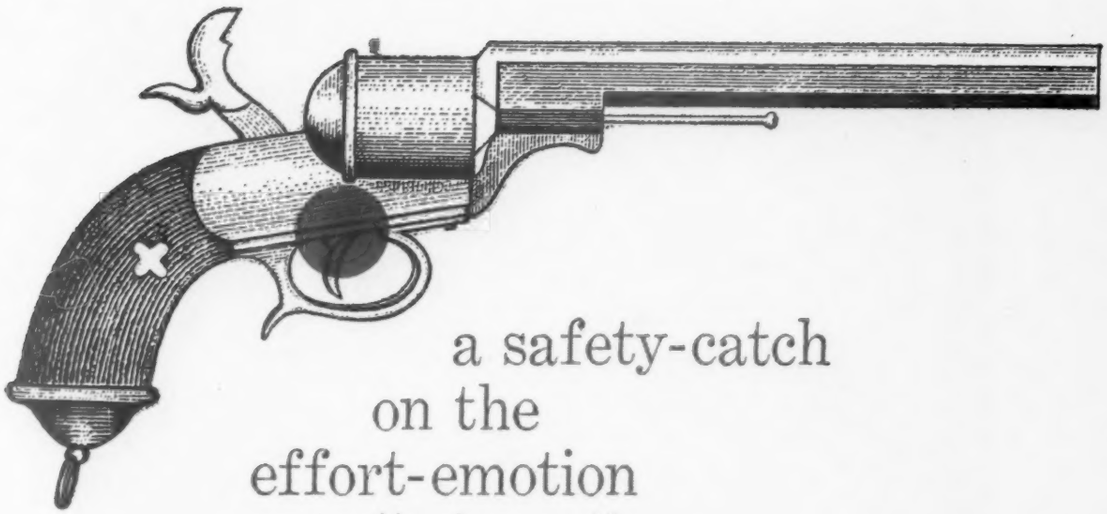
RENOGRAFIN 76%
for intravenous urography and angiography. Ampuls of 20 cc., with 1 cc. ampul for sensitivity testing. Each cc. contains 760 mg. of sodium and methylglucamine diatrizoate.

RENOGRAFIN 60%
for intravenous urography and angiography. Ampuls of 25 cc., with 1 cc. ampul for sensitivity testing. Each cc. contains 600 mg. of sodium and methylglucamine diatrizoate.

RENOGRAFIN 30%
for retrograde pyelography. Rubber-capped vials of 50 cc. Each cc. contains 299 mg. of sodium and methylglucamine diatrizoate.

"RENOGRAFIN"® IS A SQUIBB TRADEMARK

SQUIBB *Squibb Quality—the Priceless Ingredient*



a safety-catch
on the
effort-emotion
"trigger"

new

Pentraline*

vasodilator • calmative (formerly Pentrasine)

for comprehensive angina prophylaxis

PENTRALINE inhibits precipitating stimuli...helps reduce the severity and frequency of anginal attacks through the complementary action of its three components. Rauwolfia preparations and pentaerythritol tetranitrate, for example, when given together produce greater benefits than either drug used alone.[1] The reserpine alkaloid of rauwolfia also enhances the gentle sedation induced by Butisol Sodium® with mild, safe tranquilization. Thus PENTRALINE represents a comprehensive and rational approach to angina management. Plan to try PENTRALINE soon.

Each scored pink tablet contains:

Pentaerythritol Tetranitrate—10 mg.—best of the long-acting coronary vasodilators[2]

Butisol Sodium® Butabarbital Sodium—10 mg.—intermediate sedative ideally suited to long-term therapy[3]

Reserpine—0.05 mg.—effective adjunct in the management of angina pectoris[1,4]

Suggested Dosage: **One tablet four times a day before meals and at bedtime.**

Note: **PENTRALINE is not intended for treatment of acute attacks of angina pectoris, but rather for routine daily use as a preventive measure.**

References: **(1) Deitz, G. W.: Am. Pract. & Digest. Treat. *6*:1872, 1955. (2) Russek, H. I.; Zohman, B. L.; Drumm, A. E.; Weingarten, W. and Dorset, V. J.: Circulation *12*:169, 1955. (3) Dripps, R. D.: J.A.M.A. *139*:148, 1949. (4) Lewis, B. I.; Lubin, R. C.; January, L. E., and Wild, J. B.: Circulation *14*:227, 1956.**

*TRADE-MARK

15459

McNEIL LABORATORIES, INC • PHILADELPHIA 32, PA.

minor chemical changes can mean major therapeutic improvements

Medrol*

The most efficient of all anti-inflammatory steroids

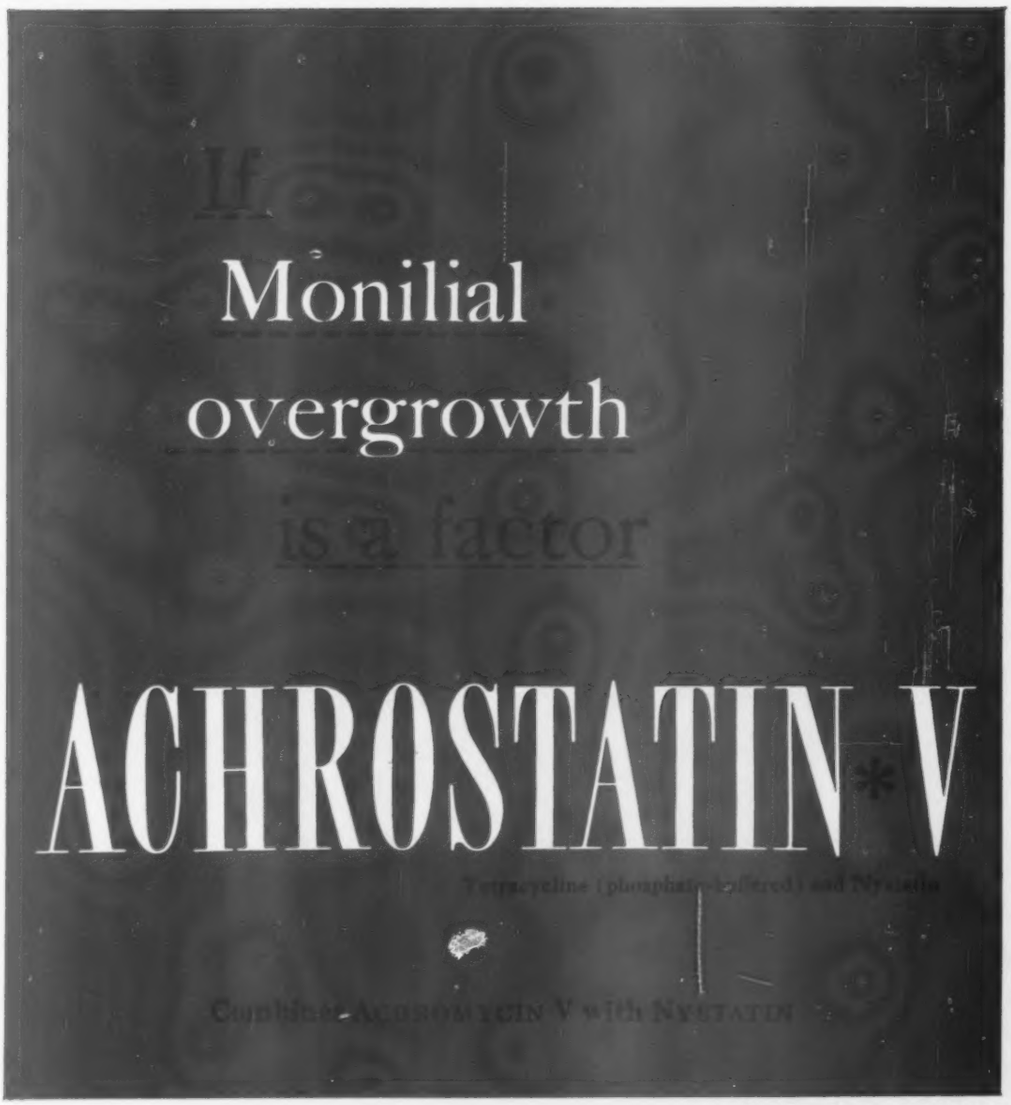
- Lower dosage (⅓ lower dosage than prednisolone)
- Better tolerated (less sodium retention, less gastric irritation)

Supplied: Tablets of 4 mg., in bottles of 30, 100 and 500.

*TRADEMARK FOR METHYLPREDNISOLONE, UPJOHN

For complete information, consult your Upjohn representative, or write the Medical Department, The Upjohn Company, Kalamazoo, Michigan.

Upjohn



ACHROSTATIN V combines ACHROMYCIN† V... the new rapid-acting oral form of ACHROMYCIN† Tetracycline... noted for its outstanding effectiveness against more than 50 different infections... and NYSTATIN... the antifungal specific. ACHROSTATIN V provides particularly effective therapy for those patients who are prone to monilial overgrowth during the protracted course of antibiotic treatment.

supplied:
ACHROSTATIN V CAPSULES contain 250 mg. tetracycline HCl equivalent (phosphate-buffered) and 250,000 units NYSTATIN.

dosage:
Basic oral dosage (6-7 mg. per lb. body weight per day) in the average adult is 4 Capsules of ACHROSTATIN V per day, equivalent to 1 Gm. of ACHROMYCIN V.

*Trademark
†Reg. U. S. Pat. Off.

Lederle LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK

your patients with generalized gastrointestinal complaints need the comprehensive benefits of

Tridal®

(DACTIL® + PIPTAL®—in one tablet)

rapid, prolonged relief throughout the G.I. tract
with unusual freedom from antispasmodic
and anticholinergic side effects

LAKESIDE

One tablet two or three times a day and one at bedtime. Each TRIDAL tablet contains 50 mg. of Dactil, the *only* brand of N-ethyl-3-piperidyl diphenylacetate hydrochloride, and 5 mg. of Piptal, the *only* brand of N-ethyl-3-piperidyl-benzilate methobromide.

14357

overwhelmingly specified
by generalists and specialists

METICORTEN®

prednisone

a standard steroid
overwhelmingly acclaimed
by

- ... **Internists** in rheumatoid arthritis, rheumatic fever and systemic lupus erythematosus
- ... **Allergists** in urticaria, angioedema, drug reactions and allergic rhinitis
- ... **Ophthalmologists** in uveitis, choroiditis and chorioretinitis
- ... **Dermatologists** in pemphigus, erythema multiforme, atopic eczemas and contact dermatoses
- ... **Chest Physicians** in bronchial asthma, pulmonary fibrosis and emphysema

and by general practitioners for virtual absence
of salt retention

METICORTEN, 1, 2.5 and 5 mg. white tablets

Schering

SCHERING CORPORATION • BLOOMFIELD, NEW JERSEY

MC-J-73117

first thought for high b.p.*

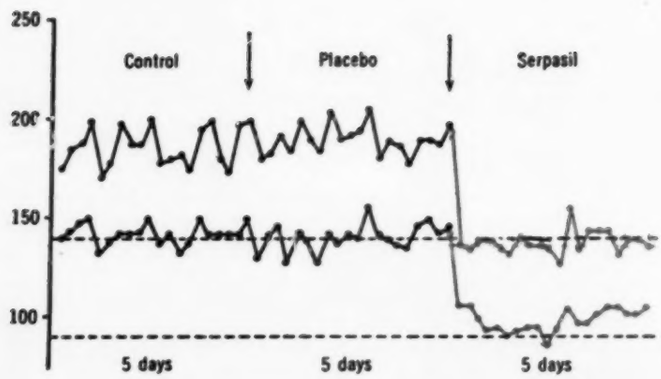


*Chart shows actual response to Serpasil in a patient with benign essential hypertension (data on request). Consider Serpasil® (reserpine CIBA) (1) alone to lower blood pressure gradually and safely in most cases of mild to moderate hypertension; (2) as a primer in severe hypertension before more potent drugs are introduced; (3) as a background agent in all grades of hypertension to permit lower dosage and thus minimize side effects of other antihypertensives.

C I B A

Three steps are necessary—

In establishing correct eating patterns

supervision by the physician[1,2,3]

a balanced eating plan[1,2,3]

selective medication[1,2,3]

Obedrin®

and the 60-10-70 Basic Plan

Following the establishment of desired eating patterns—the maintenance of the acquired habits is most important. Here, Obedrin and the 60-10-70 Plan can be valuable aids to both the physician and patient.

Obedrin provides:

- Methamphetamine for its proven anorexigenic and mood-lifting effects.
- Pentobarbital as a balancing agent, to guard against excitation.
- Vitamins B_1 and B_2 plus niacin to supplement the diet.
- Ascorbic acid to aid in the mobilization of tissue fluids.

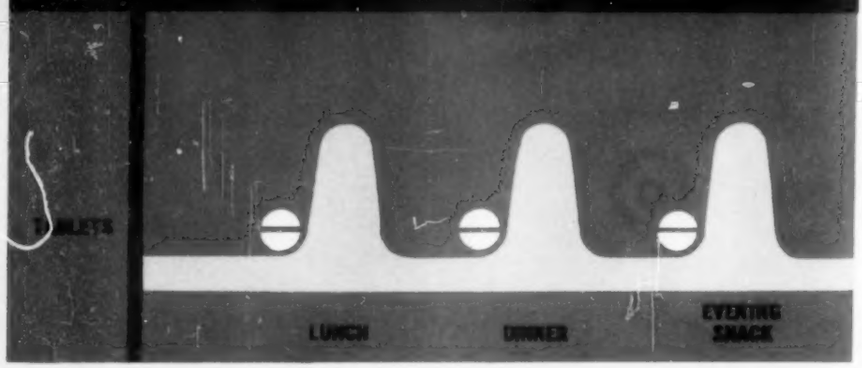
Formula:

Semoxydrine® HCl (Methamphetamine HCl)	5 mg.
Pentobarbital	20 mg.
Ascorbic Acid	100 mg.
Thiamine Mononitrate	0.5 mg.
Riboflavin	1 mg.
Nicotinic Acid	5 mg.

1. *Eisfelder, H.W.: Am Pract. & Dig. Treat. 5:778 (Oct. 1954)*
2. *Freed, S.C.: G.P. 7:63 (1953)*
3. *Sherman, R.J.: Medical Times, 82:107 (Feb. 1954)*

Bristol, Tennessee • New York • Kansas City • San Francisco THE S. E. MASSENGILL COMPANY

A flexible dosage form for predictable effect



Obedrin tablets provide a flexible dosage form which may be prescribed to depress the appetite at peak hunger periods.

The pentobarbital content assures minimal central nervous stimulation, and the 60-10-70 Basic Plan provides for a balanced food intake with sufficient protein and roughage.

Advantages of Obedrin

An effective anorexigenic agent
A flexible dosage form
Minimal central nervous stimulation
Vitamins to supplement the diet
No hazards of impaction

Obedrin®

and the 60-10-70 Basic Plan

Currently, mailings will be forwarded only at your request. Write for 60-10-70 menus, weight charts, and samples of Obedrin.

Bristol, Tennessee • New York • Kansas City • San Francisco

THE S. E. MASSENGILL COMPANY

PHENAPHEN® PLUS

Phenaphen Plus is the physician-requested combination of **Phenaphen,** plus an antihistaminic and a nasal decongestant.

Available on prescription only. Robins

each coated tablet contains:	**Phenaphen**
Phenacetin (3 gr.)	194.0 mg.
Acetylsalicylic Acid (2½ gr.)	162.0 mg.
Phenobarbital (¼ gr.)	16.2 mg.
Hyoscyamine Sulfate	0.031 mg.
plus	
Prophenpyridamine Maleate	12.5 mg.
Phenylephrine Hydrochloride	10.0 mg.

when anxiety and tension "erupts" in the G. I. tract...

IN GASTRIC ULCER

PATHIBAMATE*

Meprobamate with PATHILON® Lederle

Combines Meprobamate (*400 mg.*) the most widely prescribed tranquilizer . . . helps control the "emotional overlay" of gastric ulcer — without fear of barbiturate loginess, hangover or habituation . . . *with* PATHILON (*25 mg.*) the anticholinergic noted for its extremely low toxicity and high effectiveness in the treatment of many G.I. disorders.

Dosage: 1 tablet t.i.d. at mealtime. 2 tablets at bedtime. *Supplied:* Bottles of 100, 1,000.

*Trademark ®Registered Trademark for Tridihexethyl Iodide Lederle

Lederle

LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK

ANXIETY IS COSTLY TO THE HEART

Emotional stress is a serious threat to an already diseased heart. Thoracic muscular tension simulating cardiac pain is an added source of apprehension.

Anxiety may precipitate tachycardia, various arrhythmias, coronary insufficiency, and increased cardiac decompensation.

'Miltown' relaxes both mind and skeletal muscle. Therapeutic management with 'Miltown' (200 mg. q.i.d.) "definitely reduced nervous tension and anxiety" in all patients (80 cases), and enhanced recovery from acute cardiac episodes in many cases.*

Miltown®

THE ORIGINAL MEPROBAMATE
DISCOVERED & INTRODUCED BY
WALLACE LABORATORIES
NEW BRUNSWICK, NEW JERSEY

*Waldman, S. and Pelner, L.: Management of anxiety associated with heart disease. Am. Pract. & Digest Treat. 8:1075, July 1957.

CM-5686

"This substance [Vitamin K_1] has added greatly to the safety of anticoagulant therapy"[1]

reverse anticoagulant-induced hypoprothrombinemia

MEPHYTON®

VITAMIN K_1

the only available preparation chemically identical with naturally-occurring vitamin K_1... "has a more prompt, more potent and more prolonged effect than the vitamin K analogues"[2]

Dosage: Orally, to modify anticoagulant effects: 5 to 10 mg. initially; 15 to 25 mg. for more vigorous action. Intravenously, for anticoagulant-induced bleeding emergencies, 10 to 50 mg.; may be repeated as indicated by prothrombin time response. (Some clinicians advise their patients to keep a supply of tablets on hand at all times; if gross bleeding occurs, the patients are instructed to take 10 mg. and phone the doctor.[1])

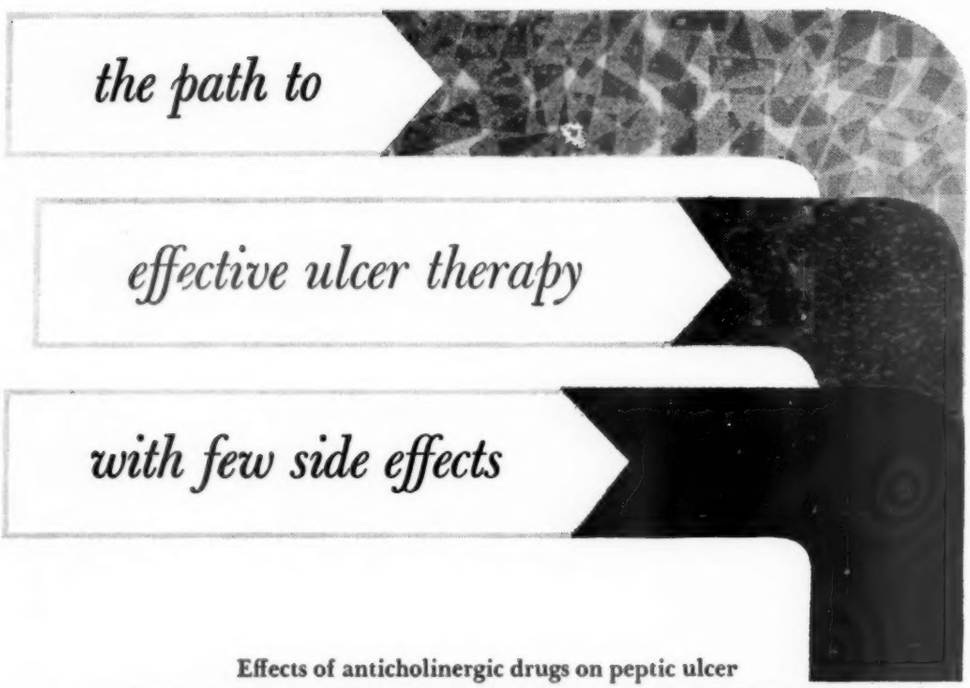
Supplied: Tablets, 5 mg., bottles of 100. Emulsion, each 1-cc. ampul contains 50 mg., boxes of 6 ampuls.

Other indications: To normalize prothrombin time—before surgery, in obstructive jaundice, hepatic disease, impaired gastrointestinal absorption, deficiency of vitamin K in the newborn, and following the administration of antibiotics, sulfonamides, and salicylates. 'Mephyton' is a valuable addition to the physician's bag for emergency use.

MSD MERCK SHARP & DOHME
DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

Mephyton is a trade-mark of MERCK & CO., INC.

1. Wright, I. S.: Early use of anticoagulants in treatment of myocardial infarction, J.A.M.A. 163: 918-921, March 16, 1957.
2. Council on Pharmacy and Chemistry: New and Nonofficial Remedies, Philadelphia, J. B. Lippincott Co., 1956, p. 505.



Effects of anticholinergic drugs on peptic ulcer

	Atropine	Anticholinergic A	Anticholinergic B	PATHILON
Daily Dose	1.6 mg.	400 mg.	120 mg.	200 mg.
No. patients and length of follow-up	37 11 mo.	27 13 mo.	16 9 mo.	21 11 mo.
Results:				
Good to excellent	51%	74%	56%	76%
Fair to poor	49%	26%	44%	24%
Recurrences:				
None	16%	22%	13%	19%
Few	46%	48%	50%	57%
Same	38%	38%	38%	24%
Complications:				
Hemorrhage	5%	7%	19%	9.5%
Perforation	0%	4%	0%	0%
Obstruction	0%	4%	0%	0%
Surgery needed	3%	4%	6%	0%
Side effects:				
Oral	38%	78%	25%	14%
Visual	11%	48%	6%	0%
Sphincter	11%	15%	0%	0%

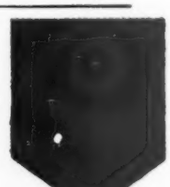
Available in three forms: tablets of 25 mg., plain (Pink) or with phenobarbital, 15 mg. (Blue), and parenteral, 10 mg./cc.—1 cc. ampuls.

Dosage: 1 or 2 tablets before each meal and at bedtime. Parenterally, 10 to 20 mg. every 6 hours.

Also available: PATHIBAMATE** Meprobamate with PATHILON LEDERLE, for gastrointestinal disorders and their "emotional overlay."

1. After Cayer, D.: Prolonged anticholinergic therapy of duodenal ulcer, Am. J. Digest. Dis. *1*:301 (July) 1956.

*Reg. U.S. Pat. Off. **Trademark

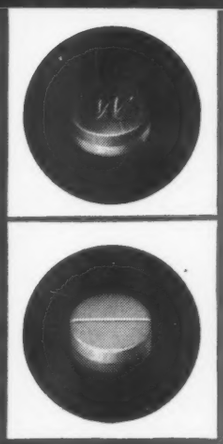


in anticholinergic therapy... weigh the benefits against the side effects

PATHILON*

Tridihexethyl Iodide LEDERLE

LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK



a **NEW** oral analgesic compound for moderate to moderately severe musculoskeletal pain

Zactirin*

Ethoheptazine Citrate with Acetylsalicylic Acid, Wyeth

*Trademark

contains the first new analgesic of its potency type in 60 years

2

ZACTIRIN tablets are equivalent in potency to ½ grain of codeine plus 10 grains of acetylsalicylic acid

effective, well-tolerated . . .
free of codeine's side-effects, free of addiction liability, free of appreciable drug tolerance

POTENTLY
ANALGESIC

SAFELY
NON-NARCOTIC

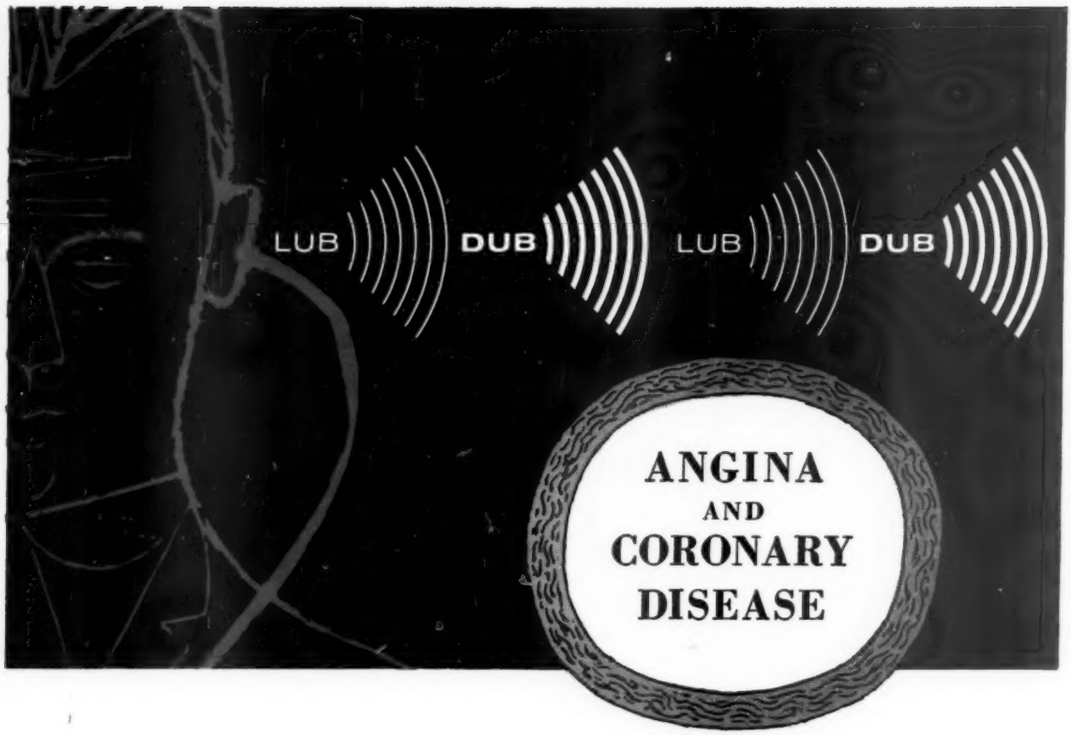
EFFECTIVELY
ANTI-INFLAMMATORY

- Low-back pain
- Pain of bursitis, synovitis, and related conditions
- Minor traumatic pain
- Subacute postoperative pain
- Postpartum abdominal or perineal pain
- Pain secondary to dental infections or dental surgery

Wyeth

SUPPLIED: Distinctive, 2-layer yellow-and-green tablets, bottles of 48. Each tablet contains 75 mg. of ethoheptazine citrate and 325 mg. (5 grains) of acetylsalicylic acid.

Comprehensive literature available on request.



The Value of Blood Cholesterol Regulation

Studies[1-4] indicate that atherosclerosis is the underlying disease process in 80-90% of Americans who had myocardial infarction or angina with abnormal EKG patterns. It is also known that patients with coronary disease frequently have elevated blood cholesterol levels.

In these studies[1-4] patients with coronary occlusion "felt better" when their blood cholesterol was reduced by diet and a special cholesterol lowering formula. Anginal symptoms abated and none had a new coronary occlusion while on this therapy.

Patients with anginal attacks treated by Schroeder[5] with a high essential fatty acid and B_6 diet noted considerable lessening of anginal attacks.

Van Gasse and Miller[6] report marked pain relief by angina patients while on linoleic acid and B_6 medication. They recommend this formula for patients with high cholesterol levels, coronary thrombosis or angina.

(1) Lobecki, T. D.: Am. J. Clin. Nutrition 3: 132, 1955.
(2) Gertler, M. M., et al.: Circulation 2: 696, 1950.
(3) Gofman, J. W., et al.: Mod. Med. 21: 119, 1953.
(4) Barr, D. P., et al.: Am. J. Med. 11: 480, 1951.
(5) Schroeder, H. A.: J. Chronic Dis. 2: 28, 1955.
(6) Van Gasse, J. J., and Miller, R. F.: A.M.A. Meeting, New York, June 3-7, 1957.

The preponderance of evidence indicates that all persons who have elevated blood cholesterol (with or without clinical evidence of disease) . . . and all persons with a condition associated with atherosclerosis (even though blood cholesterol is normal) are candidates for a cholesterol regulation program.

ARCOFAC (Armour Cholesterol Lowering Factor) was specifically formulated to lower blood cholesterol with as little as 1 dose a day . . . and at the same time allow the patient to eat a palatable, balanced and nutritious diet. ARCOFAC is the first truly practical and effective method for lowering blood cholesterol levels.

Each tablespoonful of ARCOFAC emulsion contains:

Linoleic acid	6.8 Gm.
Vitamin B_6	0.6 mg.
Mixed tocopherols (vitamin E)	11.5 mg.

Bottles of 12 fluid ounces.

Arcofac

Armour Cholesterol Lowering Factor

THE ARMOUR LABORATORIES

A DIVISION OF ARMOUR AND COMPANY • KANKAKEE, ILLINOIS

for the peak of analgesic efficiency

DILAUDID

brand of DIHYDROMORPHINONE

Dosage Forms of Dilaudid hydrochloride:
Ampules: 1cc., 2 mg. and 3 mg. each.
Hypodermic Tablets: 2, 3 and 4 mg. each.
Oral Tablets: 2.7 mg. each.
Multiple Dose Vial: 10 cc., 2 mg. Dilaudid sulfate per cc.

*Subject to Federal narcotic regulations
Dilaudid®, E. Bilhuber, Inc.

KNOLL PHARMACEUTICAL COMPANY ORANGE NEW JERSEY

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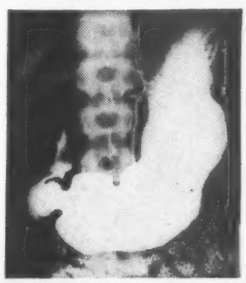
when anxiety and tension "erupts" in the G. I. tract...

IN DUODENAL ULCER



PATHIBAMATE*

Meprobamate with PATHILON® Lederle

Combines Meprobamate (*400 mg.*) the most widely prescribed tranquilizer . . . helps control the "emotional overlay" of duodenal ulcer — without fear of barbiturate loginess, hangover or habituation . . . *with* PATHILON (*25 mg.*) the anticholinergic noted for its extremely low toxicity and high effectiveness in the treatment of many G.I. disorders.

Dosage: 1 tablet t.i.d. at mealtime. 2 tablets at bedtime. *Supplied:* Bottles of 100, 1,000.

*Trademark ® Registered Trademark for Tridihexethyl Iodide Lederle

Lederle

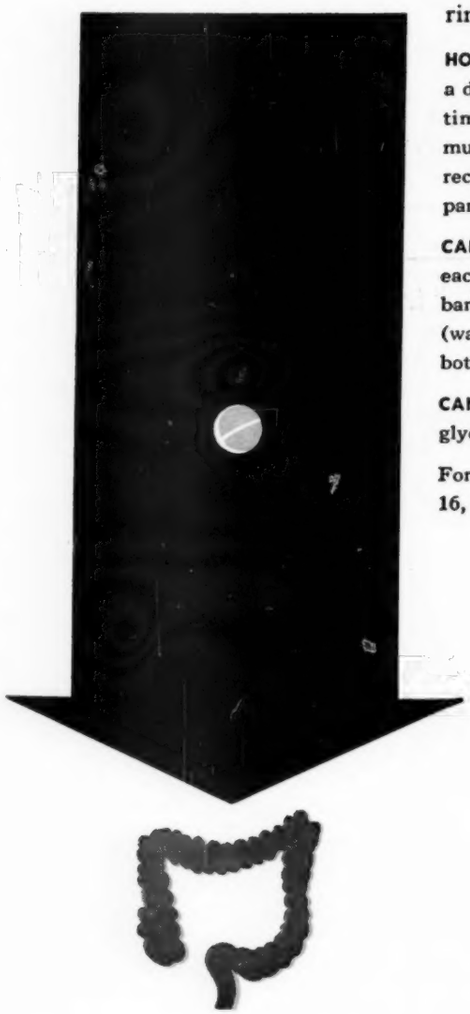
LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY, PEARL RIVER, NEW YORK

effective, selective therapy

Cantil
for the colon

for functional and organic disorders

CANTIL relieves pain, cramps, bloating...curbs diarrhea...restores normal tone and motility. Selective action focused on the colon avoids widespread interference with normal autonomic function... minimizes urinary retention, mouth dryness, blurring of vision.



HOW CANTIL IS PRESCRIBED One or two tablets three times a day preferably with meals, and one or two tablets at bedtime for patients with ulcerative colitis, irritable colon, mucous colitis, spastic colitis, diverticulitis, diverticulosis, rectospasm, diarrhea following G.I. surgery, bacillary and parasitic disorders.

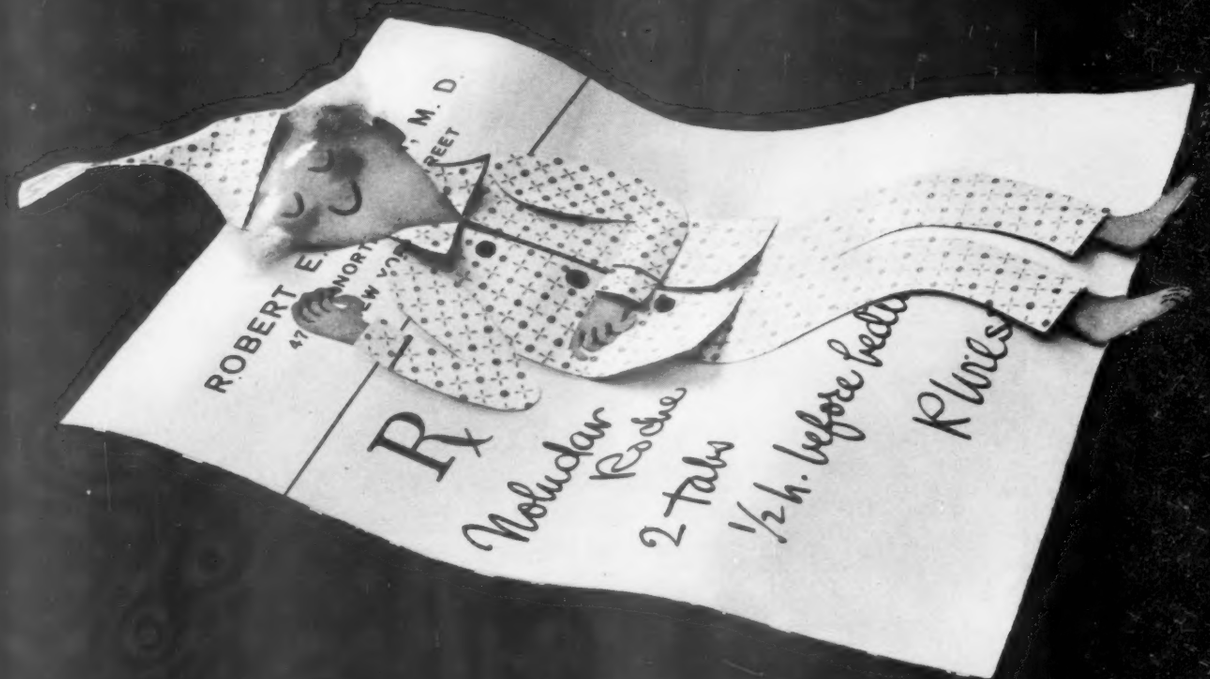
CANTIL—TWO FORMS CANTIL (plain)—25 mg. of CANTIL in each scored tablet—bottles of 100. CANTIL with Phenobarbital—25 mg. of CANTIL and 16 mg. of phenobarbital (warning: may be habit forming) in each scored tablet—bottles of 100.

CANTIL is the *only* brand of N-methyl-3-piperidyl-diphenylglycolate methobromide.

For more detailed information, request Brochure No. NDA 16, Lakeside Laboratories, Milwaukee 1, Wisconsin.

LAKESIDE

14487



MAGIC CARPET TO A GOOD NIGHT'S SLEEP

NOLUDAR® Roche

methyprylon

- At bedtime or for pre-dawn insomnia
- Non-barbiturate, relatively non-habit forming
- No morning mental haze — a ringing telephone or alarm clock easily rouses the patient at any time during sleep.
- Broad safety margin
- The physician's personal hypnotic — Noludar is advantageous when it is necessary to answer the telephone or go on a call at night.

DOSAGE:

two 200 mg tablets gently but firmly put the confirmed insomniac to sleep.

one 200 mg tablet lulls the geriatric or pediatric patient to sleep.

one 50 mg t.i.d. provides daytime sedation without drowsiness.

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc
Nutley 10, New Jersey

Roche – Reg. U.S. Pat. Off.

a new recourse
for severe
bacterial infections--
even when other
antimicrobials fail

FURADANTIN® INTRAVENOUS SOLUTION

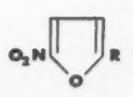
BRAND OF NITROFURANTOIN

often rapidly effective:

in systemic infections such as septicemia (bacteremia), peritonitis, and other bacterial infections as of postoperative wounds and abscesses, when the organism is susceptible to FURADANTIN; *in severe genitourinary tract* infections when the patient is unable to take FURADANTIN by mouth.

FURADANTIN I.V. has proven dramatically effective—often lifesaving—even in infections which failed to respond to other antibacterials. It has been administered to adults and children alike without serious toxic effects.

FURADANTIN I.V. solution is dissolved aseptically in a sterile diluent at room temperature, just prior to use by intravenous drip only. Full dosage instructions and discussion of indications and side effects are enclosed in each package. FURADANTIN I.V. is now available to all hospital pharmacies.

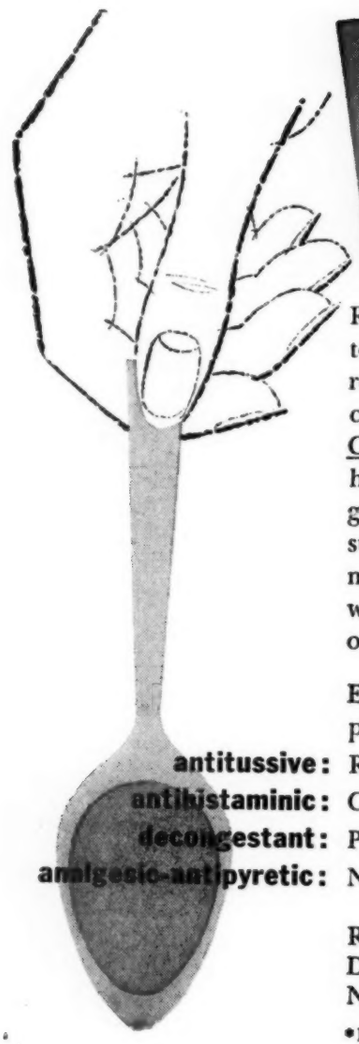


NITROFURANS—a *new* class of antimicrobials—neither antibiotics nor sulfonamides

EATON LABORATORIES, NORWICH, NEW YORK

Announcing

ROMILAR CF



the new, complete Cold Formula

Romilar CF brings new comfort and ease to your patients with colds and other respiratory disorders by providing more complete symptomatic control. Romilar CF syrup combines the benefits of an antihistaminic, a decongestant, and an analgesic-antipyretic with the effective cough suppressant action of Romilar Hydrobromide* – the non-narcotic cough specific with codeine's antitussive effect but without codeine's side effects.

Each teaspoonful (5 cc) of Romilar CF provides:

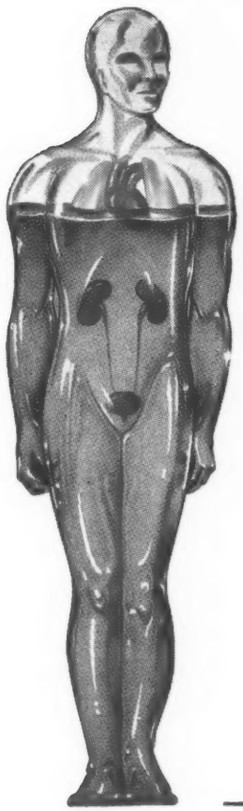
antitussive:	Romilar ® Hydrobromide*	15 mg
antihistaminic:	Chlorpheniramine Maleate	1.25 mg
decongestant:	Phenylephrine Hydrochloride	5 mg
analgesic-antipyretic:	N-acetyl-p-aminophenol	120 mg

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc
Nutley, New Jersey

*Brand of dextromethorphan hydrobromide

MERCK SHARP & DOHME

ANNOUNCES

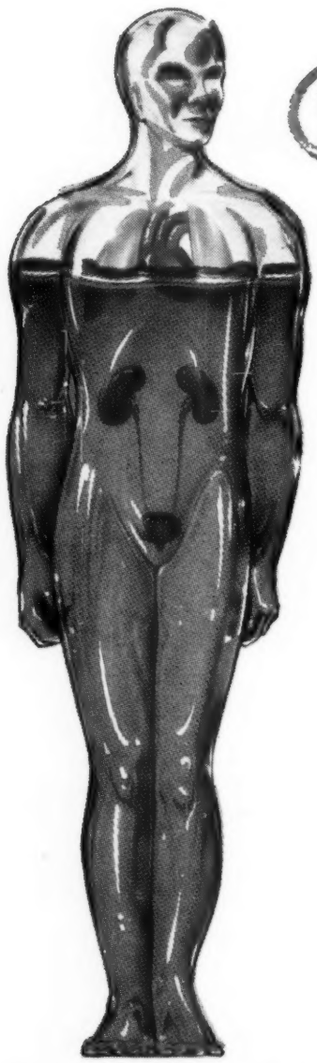


'DIURIL'

(CHLOROTHIAZIDE)

A MAJOR BREAKTHROUGH IN THE MANAGEMENT OF TWO MAJOR MEDICAL PROBLEMS

a Major Breakthrough in



'DIURIL'

(CHLOROTHIAZIDE)

'DIURIL' is a trade-mark of MERCK & CO., Inc.

Edema and Hypertension

EDEMA

1. 'DIURIL' is an entirely new, orally effective, nonmercurial agent—1 Gm. of 'DIURIL' orally being approximately equivalent to 1 cc. of mercurial I.M.
2. 'DIURIL' is ideal for initiating diuresis and for prolonged maintenance of the edema-free state.
3. 'DIURIL' promotes the balanced excretion of sodium and chloride without producing acidosis.
4. 'DIURIL' offers a rapid rising response to increased doses (within recommended dosage range).
5. 'DIURIL' is well tolerated even at maximum therapeutic doses.
6. 'DIURIL' acts rapidly (onset within 2 hours) and its moderate duration of action (6-12 hours) permits uninterrupted rest at night.
7. 'DIURIL' has no known contraindications.

any indication for diuresis is an indication for 'DIURIL'

INDICATIONS: Congestive heart failure; premenstrual edema; edema of pregnancy; renal edema—nephrosis, nephritis; cirrhosis with ascites; drug-induced edema. May be of value to relieve fluid retention complicating obesity.

DOSAGE RANGE: one 500 mg. tablet 'DIURIL' to two 500 mg. tablets 'DIURIL' once or twice a day.

HYPERTENSION

1. 'DIURIL' provides basic therapy to improve and simplify the management of hypertension.
2. 'DIURIL' often reduces dosage requirements of antihypertensive agents below the level of serious side effects.
3. 'DIURIL', added to the regimen, is often effective in controlling the blood pressure of even highly resistant cases of hypertension.
4. 'DIURIL' maintains its effectiveness even during prolonged therapy.
5. 'DIURIL' smooths out blood pressure fluctuations.
6. 'DIURIL' is orally administered with simple dosage schedules.

INDICATIONS: Hypertension of all degrees of severity.

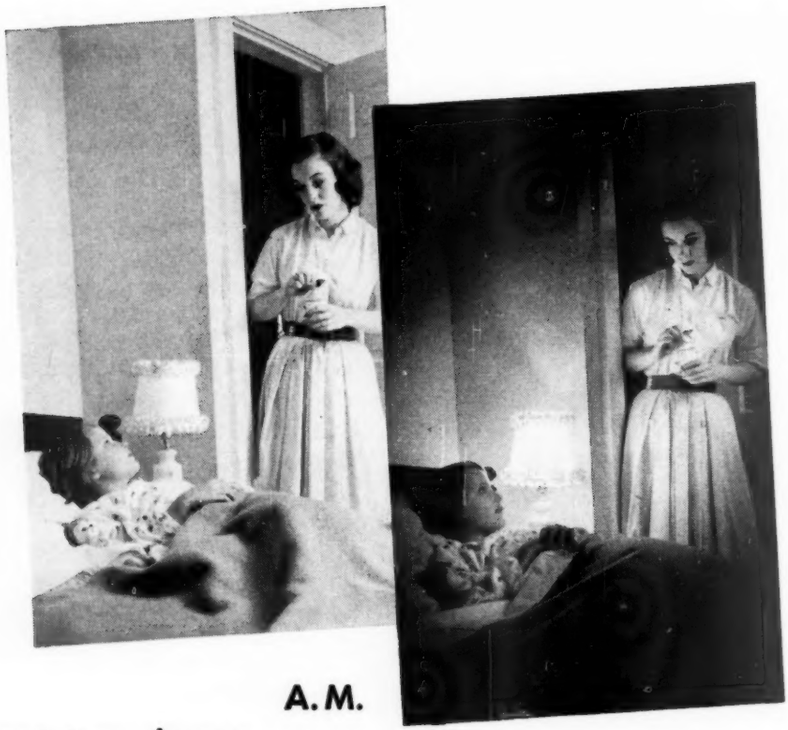
DOSAGE RANGE: one 250 mg. tablet 'DIURIL' two times daily to one 500 mg. tablet 'DIURIL' three times daily.

MSD

MERCK SHARP & DOHME
Division of MERCK & CO., Inc., Philadelphia 1, Pa.

SUPPLIED: 250 mg. and 500 mg. scored tablets of 'DIURIL' (Chlorothiazide); bottles of 100 and 1,000.

BIBLIOGRAPHY: Baer, J. E. *et al.: Fed. Proc.* **16**:278, (March) 1957; Beyer, K. H. *et al.: Fed. Proc.* **16**:282, (March) 1957; Ford, R. V. *et al.: M. Rec. & Ann.* **51**:376, (April) 1957; Ford, R. V. *et al.: Arch. Int. Med.* **100**:582, (October) 1957; Ford, R. V. *et al.: Antibiotic Med. & Clin. Therapy* (in press); Moyer, J. H. *et al.: Proc. Soc. Exper. Biol. & Med.* **95**:529, (July) 1957; Novello, F. C. and Sprague, J. M.: *J. Am. Chem. Soc.* **79**:2028, (April 20) 1957; Russo, H. F. *et al.: Fed. Proc.* **16**:333, (March) 1957; Hollander, W. and Wilkins, R. W.: *Boston Med. Quart.* **8**:69, (Sept.) 1957; Freis, E. D. *et al.: J.A.M.A.* (in press); Finnerty, F. A.: *N. Y. State J. Med.* **57**:2957, (Sept. 15) 1957; Freis, E. D. and Wilson, I. M.: *Med. Ann. District of Columbia* **26**:468, (Sept.) 1957; Freis, E. D. *et al.: Circulation* (in press).



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Just two doses—

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LIPO GANTRISIN ROCHE ®

. . . and you can be sure that your patients will have prompt, lasting plasma and urine levels.

Lipo Gantrisin is the ideal pediatric form of Gantrisin, in which 2 doses a day are sufficient to combat most urinary and systemic infections of non-viral – non-rickettsial origin.

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Lipo Gantrisin® Acetyl—brand of acetyl sulfisoxazole in a homogenized mixture

NEW...

A more potent, more effective, lower-dose antidiarrheal

With a powerful new adsorbent—having five times the adsorbent action of kaolin

With two synergistic antibiotics—having a more effective antidiarrheal spectrum (with systemic absorption so minimal as to preclude toxicity)

With increased potency—for greater therapeutic effectiveness, smaller dose

With refreshing taste—for acceptability in all age groups

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Dihydrostreptomycin Sulfate, Polymyxin B Sulfate, and Pectin with Clorsorb* (Activated Attapulgite, Wyeth) in Aluminum Gel

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Supplied: Bottles of 8 fl. oz.

"PROOF IN PRACTICE"

a study of 12,880 hypertensive patients

No. of Patients	Results	Percent
3,929	excellent	30.5%
6,393	good	49.6%
1,535	fair	11.9%
596	unsatisfactory	4.6%
427	side effects	3.3%

The tabulations at the left are from the recently completed study on cryptenamine (Unitensen) in which 12,880 patients and 1,384 physicians participated. Evaluation of the drug was based on experience in everyday private practice.

A summary of the "proof in practice" study is available upon request from the Medical Director of Irwin, Neisler & Co.

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Each Unitensen tablet contains cryptenamine (tannates) 2.0 mg.

UNITENSEN-R®

Each Unitensen-R tablet contains cryptenamine (tannates) 1.0 mg., Reserpine 0.1 mg.

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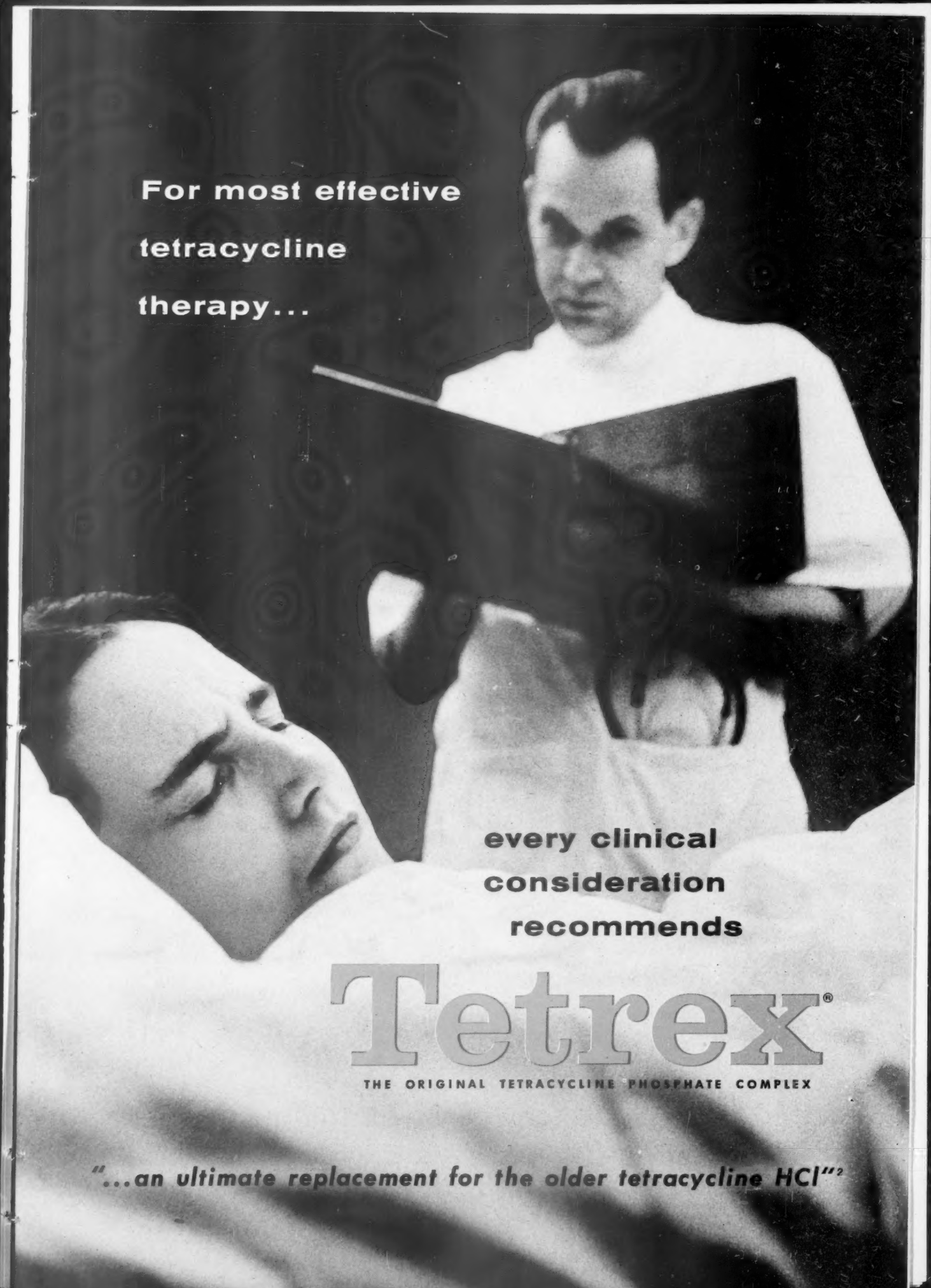
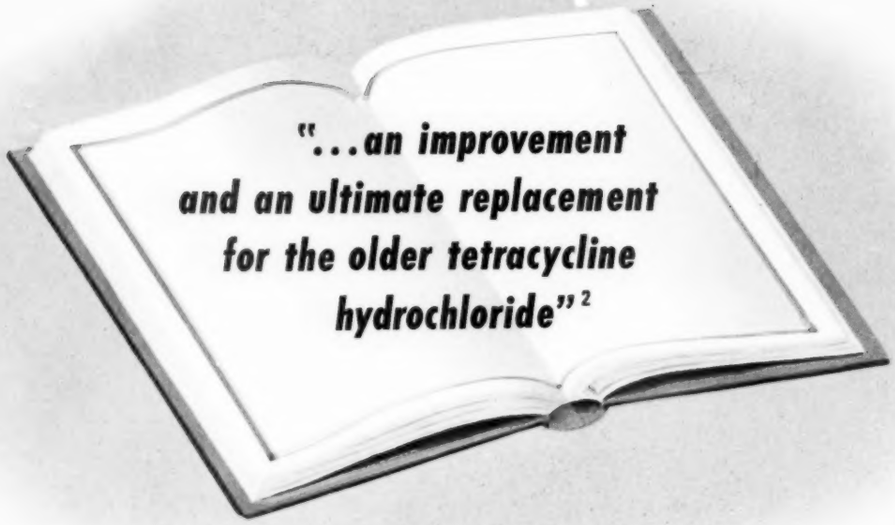

For most effective
tetracycline
therapy...
every clinical
consideration
recommends
Tetrex®
THE ORIGINAL TETRACYCLINE PHOSPHATE COMPLEX
"...an ultimate replacement for the older tetracycline HCl"[2]



Tetrex®

U.S. PAT. NO. 2,791,609

THE ORIGINAL TETRACYCLINE PHOSPHATE COMPLEX

- *Significant tetracycline serum levels for 24 hours on a single intramuscular dose*[3,6]

- *Faster, higher, more prolonged tetracycline serum levels for earlier and more certain control of infection*[1,2,5,7-13]

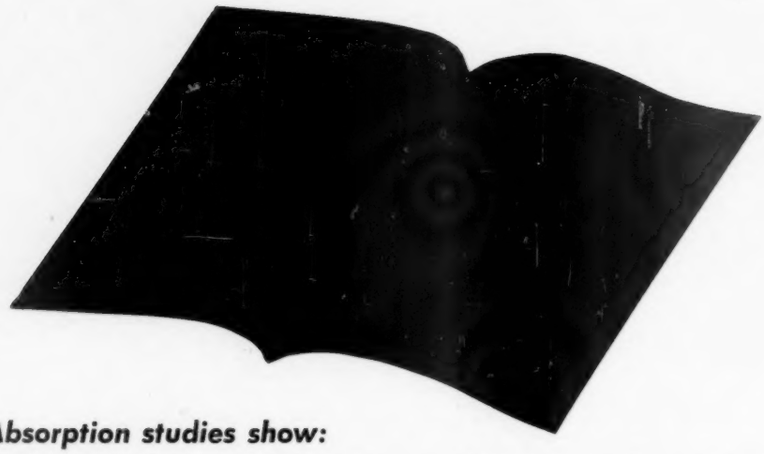
- *A single, pure antibiotic (not a mixture)*

- *Clinically "sodium-free"*[2,7]

- *Equally effective orally, b.i.d. or q.i.d.*[2,7,9]

- *A convenient dosage form for every member of the family*

Bristol

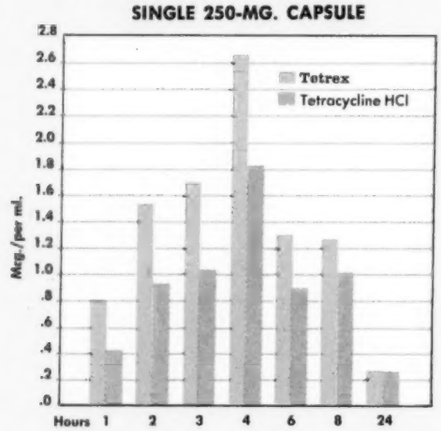


Absorption studies show:

Faster, higher, more prolonged tetracycline serum levels

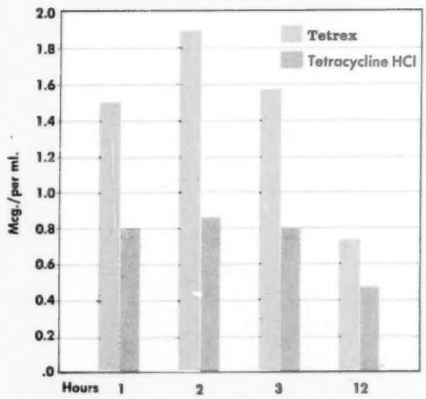
After oral administration—

SINGLE 250-MG. CAPSULE



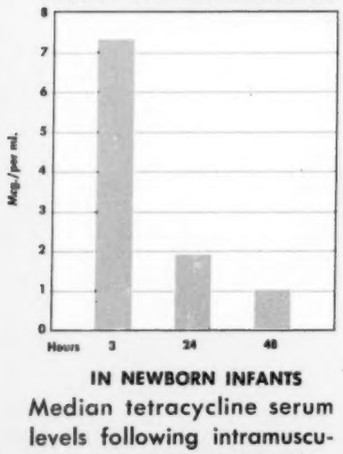
A composite of three separate absorption studies: serum levels "substantially higher"[2] or "markedly higher"[13] than with tetracycline HCl, and absorption "about twice as efficient."[5]

TWO 250-MG. CAPSULES



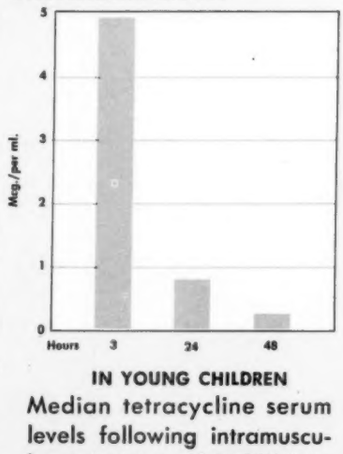
Average serum levels after administration of a single 500-mg. dose: "approximately twice as great" as those of a group receiving an equivalent dose of tetracycline HCl.[11]

After intramuscular administration—



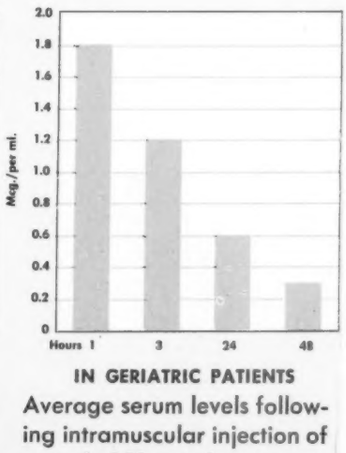
IN NEWBORN INFANTS

Median tetracycline serum levels following intramuscular injection of TETREX in newborn infants.[6] Dose: 25 mg./Kg. "Therapeutic" levels for at least 24 hours.



IN YOUNG CHILDREN

Median tetracycline serum levels following intramuscular injection of TETREX in young children.[6] Dose: 15 to 25 mg./Kg. "Surprisingly high" levels at 3 and 24 hours.



IN GERIATRIC PATIENTS

Average serum levels following intramuscular injection of a single 250-mg. dose in geriatric patients.[3] "Adequate" amounts of tetracycline activity present after 24 hours.

Rx

***"...an improvement and an ultimate replacement for the older tetracycline hydrochloride"*[2]**

Tetrex®

THE ORIGINAL TETRACYCLINE PHOSPHATE COMPLEX

Clinically proven—It has been determined that "blood concentrations correlate well with clinical response."[4] In clinical studies, the assurance that TETREX "would be more effective [than tetracycline HCl] in treating infections due to susceptible organisms"[11] has been fully confirmed.

Typically, when TETREX was administered to 686 patients in two studies, "all patients infected with tetracycline-sensitive organisms responded satisfactorily to therapy."[1,2]

A "remarkably low incidence of side reactions"[1] has been reported. In four studies involving 480 patients, for instance, side effects were so negligible as to require withdrawal of therapy in only 2 cases.[1,7,9,10]

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A suitable TETREX dosage form for every member of the family

TETREX Capsules

Tetracycline phosphate complex—each capsule equivalent to 250 mg. tetracycline HCl activity.

TETREX Pediatric Capsules

Tetracycline phosphate complex—each capsule equivalent to 100 mg. tetracycline HCl activity.

TETREX Intramuscular '250' with Xylocaine*

Tetracycline phosphate complex—each vial equivalent to 250 mg. tetracycline HCl activity.

TETREX Intramuscular '100' with Xylocaine*

Tetracycline phosphate complex—each vial equivalent to 100 mg. tetracycline HCl activity.

*® of Astra Pharm. Prod. Inc. for lidocaine

TETREX Syrup

Tetracycline (phosphate buffered) Syrup — each 5-cc. tsp. equivalent to 125 mg. tetracycline HCl activity.

TETREX Pediatric Drops

Tetracycline (phosphate buffered) Syrup — each cc. equivalent to 100 mg. tetracycline HCl activity.

TETREX-APC with BRISTAMIN® Capsules

Tetracycline phosphate complex — each capsule equivalent to 125 mg. tetracycline HCl activity, plus phenyltoloxamine and APC.

Available for your prescription at all leading pharmacies

References:

1. Cronk, G. A., and Naumann, D. E.: Ant. Med. & Clin. Ther. 4:166, 1957. 2. Cronk, G. A., Naumann, D. E., and Casson, K.: Fifth Annual Symposium on Antibiotics, Washington, D.C., Oct. 2-4, 1957. 3. Dube, A. H.: *Ibid.* 4. Flippin, H. F., and Eisenberg, G. M.: Antimicrobial Therapy in Medical Practice, Davis, Philadelphia, 1955, p. 55. 5. Kaplan, M. A., Dickison, H. L., Hubel, K. A., and Buckwalter, F. H.: Ant. Med. & Clin. Ther. 4:99, 1957. 6. Portney, B., Draper, T., and Wehrle, P. F.: Fifth Annual Symposium on Antibiotics, Washington, D.C., Oct. 2-4, 1957. 7. Prigot, A., Shidlovsky, B. A., and Felix, A. J.: Ant. Med. & Clin. Ther. 4:487, 1957. 8. Pulaski, E. J., and Isokane, R. K.: Ant. Med. & Clin. Ther. 4:408, 1957. 9. Putnam, L. E.: Ant. Med. & Clin. Ther. 4:470, 1957. 10. Rein, C. R., and Fleischmajer, R.: Ant. Med. & Clin. Ther. 4:422, 1957. 11. Shidlovsky, B. A., Prigot, A., Maynard, A. de L., Felix, A. J., and Hjelt-Harvey, I.: Fifth Annual Symposium on Antibiotics, Washington, D.C., Oct. 2-4, 1957. 12. Trafton, H. M., and Lind, H. E.: *Ibid.* 13. Welch, H., Lewis, C. N., Staffa, A. W., and Wright, W. W.: Ant. Med. & Clin. Ther. 4:215, 1957.

IN URINARY TRACT INFECTIONS

Soluble • Safe • Specific Sulfonamide

High solubility, complete absorption, minimal acetylation, and negligible penetration into red blood cells ensure rapid and effective bacteriostatic action with minimal side effects at site of infection. Ideal for long term therapy.

direct/effective

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Brand of sulfamethizole

when analgesia is also desired

"THIOSULFIL"®-A

Brand of sulfamethizole with phenylazo diamino-pyridine HCl

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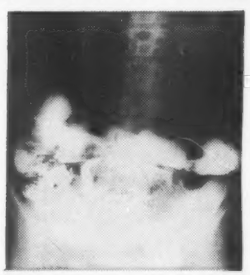
when anxiety and tension "erupts" in the G. I. tract...

IN ILEITIS



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Meprobamate with PATHILON® Lederle

Combines Meprobamate (*400 mg.*) the most widely prescribed tranquilizer . . . helps control the "emotional overlay" of ileitis — without fear of barbiturate loginess, hangover or habituation . . . *with* PATHILON (*25 mg.*) the anticholinergic noted for its extremely low toxicity and high effectiveness in the treatment of many G.I. disorders.

Dosage: 1 tablet t.i.d. at mealtime. 2 tablets at bedtime. *Supplied:* Bottles of 100, 1,000.

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AN
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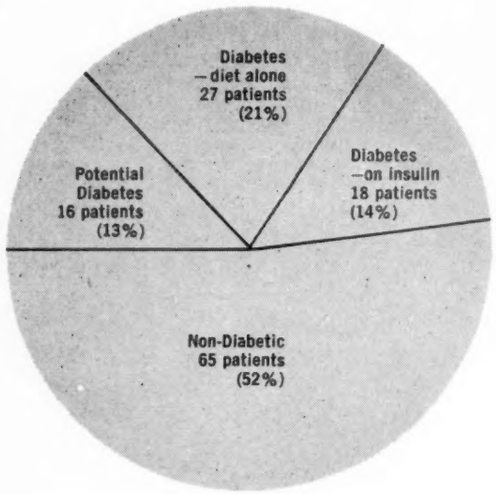
CLINIQUICK

CLINICAL BRIEFS FOR MODERN PRACTICE

DIABETES FOLLOWING TRANSIENT GLYCOSURIA*



should a non-diabetic,
transient glycosuria ever be
considered unimportant?

Never. A patient showing even a mild transient glycosuria should be observed for years as a diabetic suspect.*

Ultimate diagnosis on 126 patients with a previous transient mild glycosuria. Twenty diabetics were discovered 5-10 years after a recorded glycosuria—10 diabetics after more than 10 years.*

*Murphy, R.: Connecticut M. J. *21*:306, 1957.

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the STANDARDIZED urine-sugar test
for reliable quantitative estimations

- full color calibration, clear-cut color changes
- established "plus" system covers entire critical range
- standard blue-to-orange spectrum long familiar to diabetics
- unvarying, laboratory-controlled color scale

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PRENATAL CAPSULES **LEDERLE**

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Your prenatal and lactating patients get improved nutritional support, plus greater comfort and convenience—all at no extra cost—when you prescribe newly developed FILIBON Prenatal Capsules.

NEW better tolerated source of iron—ferrous fumarate—helps avoid gastric upset

NEW non-inhibitory intrinsic factor assures greater B_{12} absorption to meet increased requirements

NEW more comprehensive formulation . . . includes *phosphorus-free* calcium, Vitamins K and B_6, and important minerals and trace elements

NEW Reminder Jar . . . designed to be kept on the dining table where her vitamins won't be forgotten

Each capsule contains:

Vitamin A	4,000 U.S.P. Units
Vitamin D	400 U.S.P. Units
Thiamine Mononitrate (B_1)	3 mg.
Pyridoxine (B_6)	1 mg.
Niacinamide	10 mg.
Riboflavin (B_2)	2 mg.
Vitamin B_{12}	2 mcgm.
Ascorbic Acid (C)	50 mg.
Vitamin K (Menadione)	0.5 mg.
Folic Acid	1 mg.
Ferrous Fumarate	90 mg.
Iron (as Fumarate)	30 mg.
Intrinsic Factor	5 mg.
Fluorine (as CaF_2)	0.015 mg.
Copper (as CuO)	0.15 mg.
Iodine (as KI)	0.01 mg.
Potassium (as K_2SO_4)	0.835 mg.
Manganese (as MnO_2)	0.05 mg.
Magnesium (as MgO)	0.15 mg.
Molybdenum (as $Na_2MoO_4 \cdot 2H_2O$)	0.025 mg.
Zinc (as ZnO)	0.085 mg.
Calcium Carbonate	575 mg.

Dosage: one or more capsules daily.
Supplied: attractive, re-usable bottles of 100 capsules.

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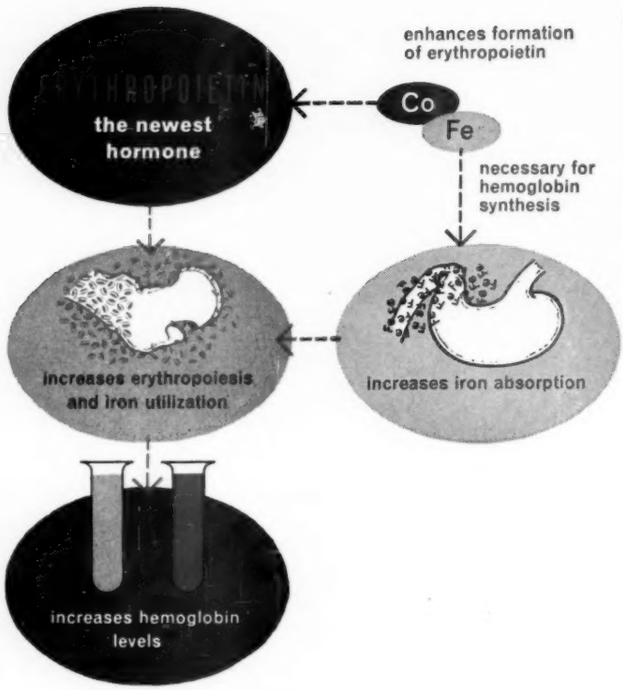

NEW RESEARCH ON ERYTHROPOIETIN EXPLAINS CLINICAL SUPERIORITY OF

RONCOVITE®-mf

(MODIFIED FORMULA)

IN THE COMMON ANEMIAS

Elucidation of the action of erythropoietin—the erythropoietic hormone—provides a clear explanation for the observations of Holly,[1] Ausman,[2] Tevetoglu[3] and many others who have reported that in the common anemias cobalt-iron therapy results in a clinical response superior to that produced by iron alone.

Increased Iron Absorption and Utilization—Recent investigations show that cobalt enhances the formation of erythropoietin.[4,5] This hormone increases the rate of production of new red cells which, in turn, increases the rate of both iron utilization by the marrow and iron absorption from the intestine.[6]

Clinical Application—In simple *iron deficiency anemia*, 89% of patients treated with Roncovite exceeded 12 Gm. of hemoglobin per 100 cc., while only 33% of the same patients treated with iron alone for a comparable period reached this level.[2] In *anemia of pregnancy*, 98.2% of Roncovite-treated patients maintained their hematologic status; 63.8% delivered with a hemoglobin of 13 Gm. per 100 cc. or more.[1] In *anemia of infancy and childhood* an average hemoglobin level of only 8.7 Gm. per 100 cc. was attained with iron alone while the same patients subsequently reached an average hemoglobin level of 11.6 Gm. per 100 cc. with Roncovite.[3]

Roncovite-MF is the new therapeutic agent based on erythropoietin formation which translates this new research into the practical utility of full iron effectiveness with greatly decreased, better tolerated iron dosage.

Each enteric-coated, green tablet contains:

Cobalt chloride, 15 mg.
Ferrous sulfate exsiccated, 100 mg.

Maximum adult dosage:

one tablet after each meal and at bedtime.
Supplied: Bottles of 100 tablets.

Bibliography available on request.

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CINCINNATI 3, OHIO

In Arthritic-Rheumatic Disorders

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	OVER **prednisolone alone** (or other corticoids)	OVER **corticoid-salicylate combinations**	OVER **other corticoid-tranquilizer combinations**
Emotional Stabilization	...**ATARAXOID** includes control of fear, anxiety	...**ATARAXOID** includes control of fear, anxiety	...only **ATARAXOID** provides the unique, specific, consistently effective tranquilizer, ATARAX
Clinical Control	...tranquilization enhances prednisolone effect for superior improvement	...tranquilization eases muscle tension (relieving aching and stiffness) precludes anxiety-induced flare-ups ...enhanced corticoid control frequently superior	...tranquilizer enhancing effect is more consistent ...established by outstanding results in 94% of 919 cases*
Dosage Levels	...corticoid requirements are frequently reduced by 25-50%	...corticoid maintenance levels compare favorably; often lower	...tranquilizer dosage levels are the lowest ...more consistent tranquilization often permits lower corticoid dosage
Toleration	...corticoid side effects are significantly reduced or eliminated	...no salicylate side effects ...reduced corticoid side effects compare favorably	...tranquilizer control is the safest—and free of mental "fogging" ...reduction of corticoid complications more consistent
Patient Management	...tranquilization greatly facilitates cooperation	...tranquilization greatly facilitates cooperation	...more consistent, uncomplicated tranquilization means better cooperation

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Ataraxoid 5.0
scored green tablets, 5.0 mg. prednisolone and 10 mg. hydroxyzine hydrochloride; bottles of 30 and 100

Ataraxoid 2.5
scored blue tablets, 2.5 mg. prednisolone and 10 mg. hydroxyzine hydrochloride; bottles of 30 and 100

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for greater flexibility of dosage

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**Individual Case Reports, Chas. Pfizer & Co., Inc.*

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with safer combination therapy

"objective relief...gratifying"

Rauvera—the combination of alseroxylon and alkavervir—is much more effective than either drug alone. This combination produces "no postural hypotension, no organ toxicity, and no sensitization reactions. Tolerance does not develop on prolonged administration...hypotensive action is steady and prolonged and persists over the entire twenty-four hours.[1]" Rauvera therapy can be continued over long periods of time.

"subjective relief...even more so"[1]

Alseroxylon and alkavervir "when combined produce mutual reinforcement so that...more severe cases respond," yet "side effects are minimal."[2] Most patients feel better, are less tired and are free from headaches.[3] Anxiety and tension are relieved...pulse rate slowed...such symptoms as "heart consciousness," tinnitus, vertigo, giddiness and insomnia disappear rapidly —leaving a calm and relaxed patient.

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1. Bandig, A.: New York State J. M. 66:2523, 1956. 2. La Barbera, J. F.: Med. Rec. and Ann. 50:242, 1956. 3. Gilchrist, A. R.: Brit. M. J. No. II:1011, 1956.

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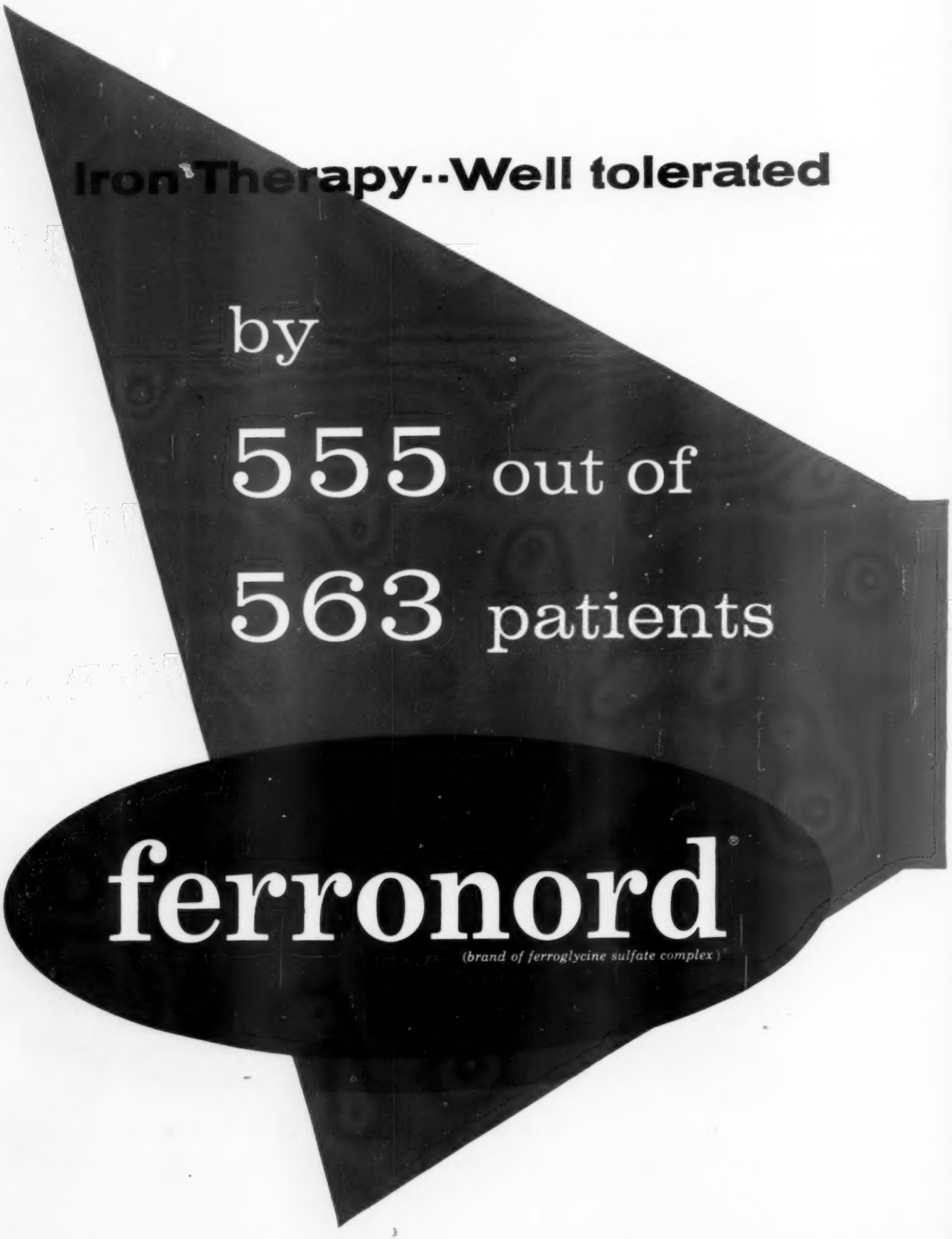
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1. Frohman, I. P.; Pomeranze, J.; Rummel, W.; Kircher, R. F.; Clancy, J. B.; Dwyer, T. A.; Wagner, H.; O'Brien, T. E.; Curley, R. T.; Jörgensen, G.; Onorato, R. R.; Ira, F.; Lee, Jr., J. G.; Gorla, W. O.; White, R. N.; Gadek, R. J.; Remy, D.: Scientific Exhibit, 6th International Congress of Hematology, Boston, Mass., August, 1956.
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*Pat. Pending. α-aminoacetic-ferrous sulfate complex, exsiccated

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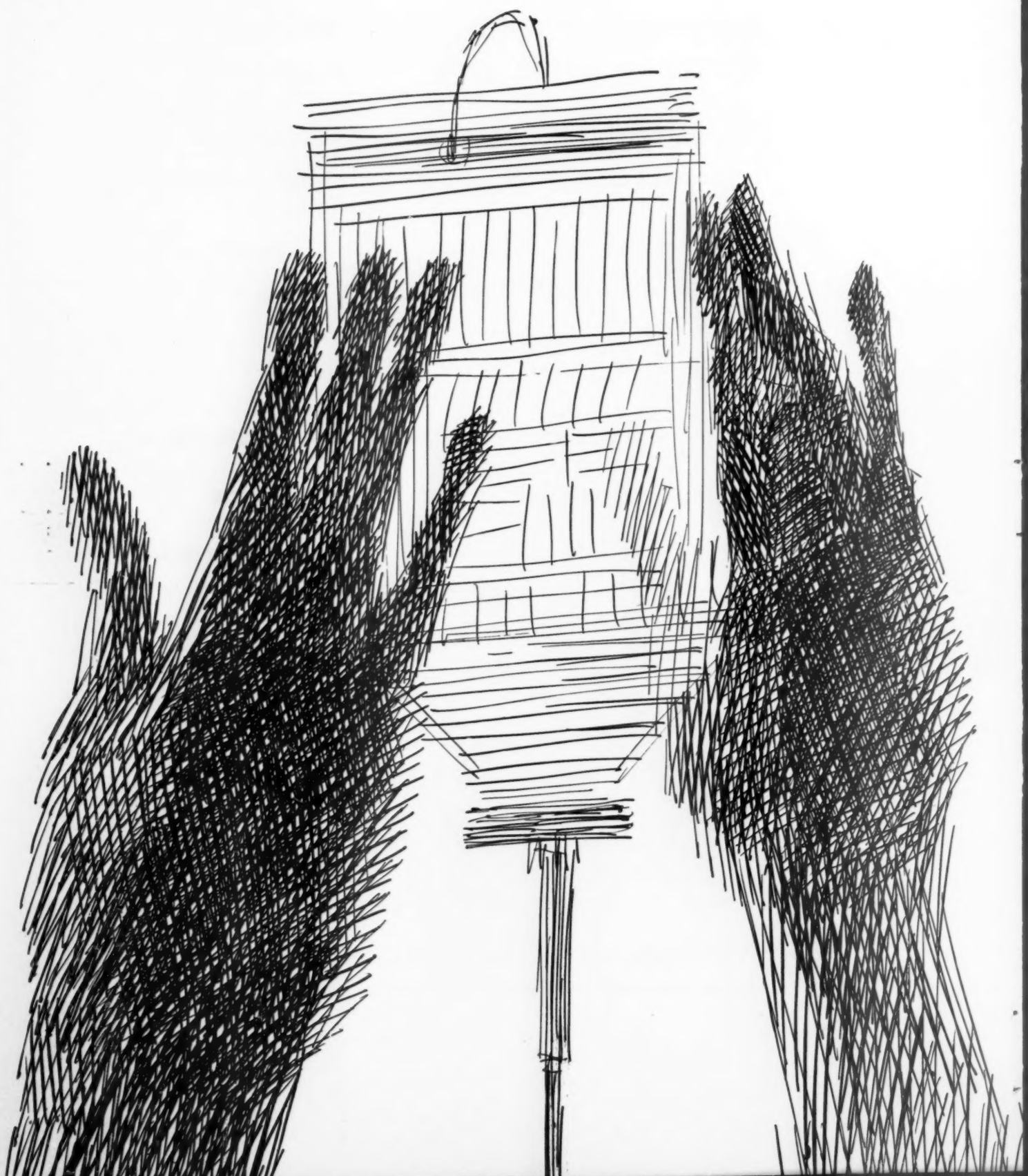

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1) *successful short-term therapy for acute or subacute endocarditis*

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1. Wright, W. T. Jr.; Pokorny, C., and Foster, T. L.: J. Kansas M. Soc. 57:410, 1956.
2. Terman, L. A.: Illinois M.J. 3:67, 1957.
3. Moyer, J. H.; Dennis, E., and Ford, R.: Arch. Int. Med. 96:530, 1955.

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HYPOTHYROIDISM

a single syndrome with a host of symptoms

Thyroid secretion acts on every system, organ, tissue and cell of the body. It controls the general metabolism; promotes growth and development; affects protein, carbohydrate and fat metabolism; helps regulate water and electrolyte balance; takes an active part in maintaining normal condition and function of the skin and circulatory, digestive, muscular, nervous and reproductive systems.

Hypothyroidism (which may range from slight to total deficiency) is manifested by an astounding number and variety of symptoms:

1 *Low BMR / Cold extremities / Elevated serum cholesterol / Slow pulse rate / Lack of energy / Sensitivity to cold / Overweight*

2 *Dry, falling hair*

3 *Somnolence / Chronic fatigue / Failing memory / Inability to concentrate / Delayed reflexes / Chronic headache / Irritability / Frank psychosis / Paresthesia*

4 *Chronic colds*

5 *Dry, coarse skin*

6 *Anemia*

7 *Sodium and water retention / Nonpitting edema / Diminished diuresis / Albuminuria*

8 *Achlorhydria / Flatulence / Constipation*

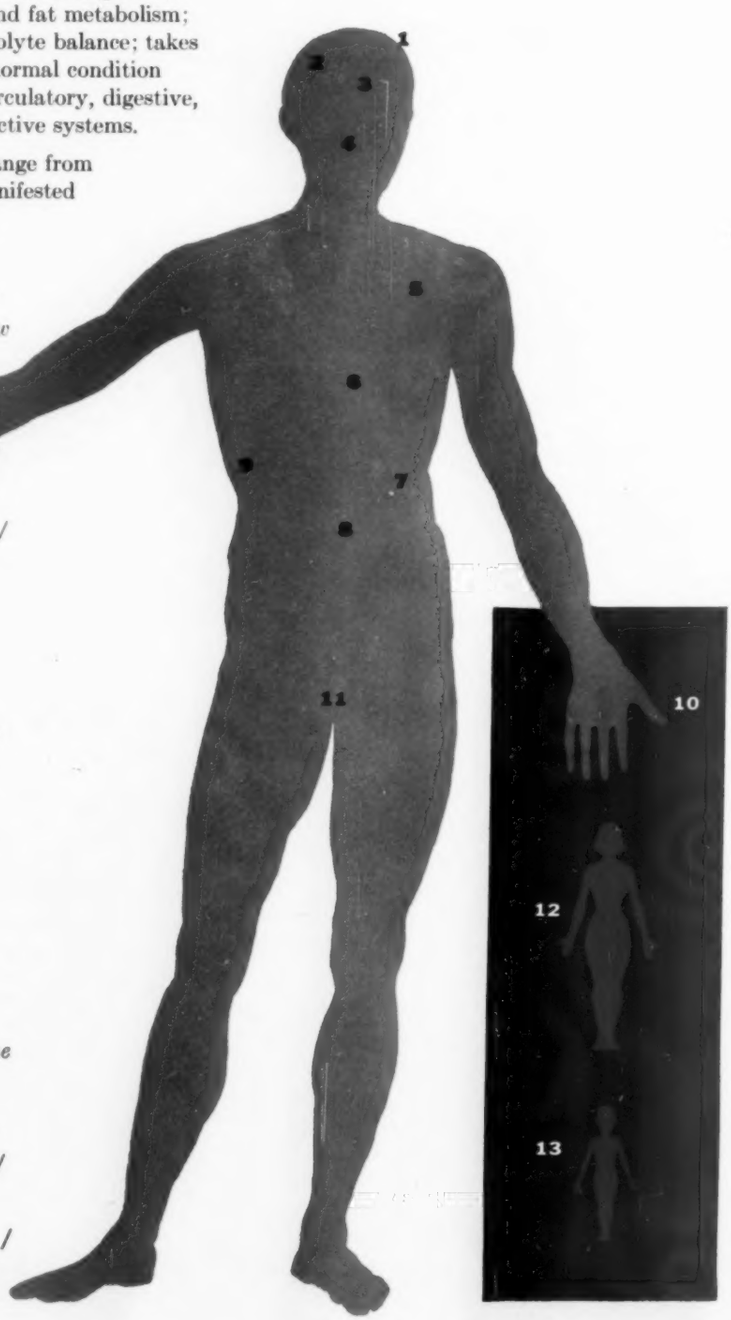
9 *Flabby muscles / Backache / Arthralgia / Myalgia*

10 *Brittle nails*

11 *Decreased libido / Infertility / Impotence*

12 *Amenorrhea / Dysmenorrhea / Menorrhagia / Functional uterine bleeding / Habitual abortion / Sterility / Failure of lactation*

13 *Stunted growth / Macroglossia / Umbilical hernia / Yellow skin / Delayed dentition / Apathy / Hoarseness / Delayed skeletal maturation / Mental retardation / Delayed sexual development / Cretinism*



DEFINITION: SUBCLINICAL HYPOTHYROIDISM (METABOLIC INSUFFICIENCY)—Recently a new name—"metabolic insufficiency"—has been applied to cover such symptoms as fatigue, lassitude, decreased mental alertness, dry skin and hair, brittle nails, inability to lose weight and high cholesterol blood levels. These are the familiar symptoms of subclinical hypothyroidism.

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PROLOID® WHENEVER THYROID IS INDICATED—Since hypothyroidism is often a lifetime deficiency, many patients must take thyroid for the rest of their lives. Long-term treatment necessitates a simple, safe, economical therapy. Proloid (purified thyroglobulin) provides the total thyroid complex. It contains all fractions of thyroid secretion in their natural physiologic ratio. Proloid provides economical and *complete* substitution therapy. It affects all the parameters of thyroid function and its effect is gradually cumulative rather than precipitous. It is also free of side effects. Because Proloid is *doubly* assayed, biologically (in test animals) as well as chemically, the clinician is assured of a smooth predictable clinical response.

Daily dose of Proloid
Proloid is prescribed in the same dosage as ordinary thyroid. The conversion table below is for convenience in calculating dosage whenever thyroid therapy is indicated to replace triiodothyronine in the long-term treatment of subclinical hypothyroidism.
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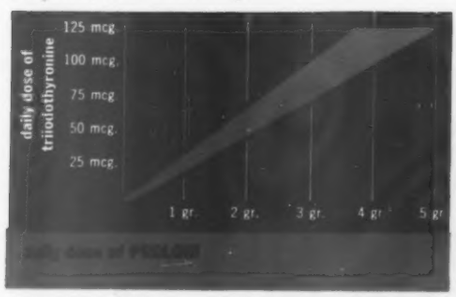

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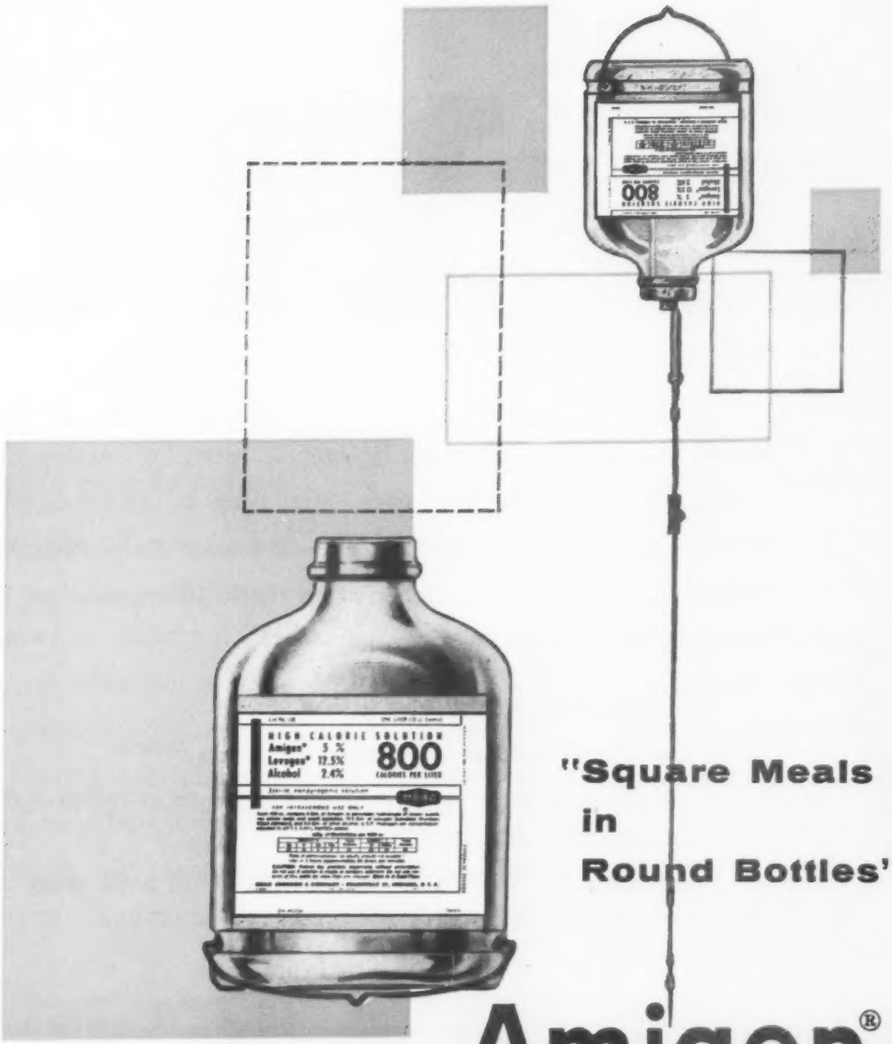

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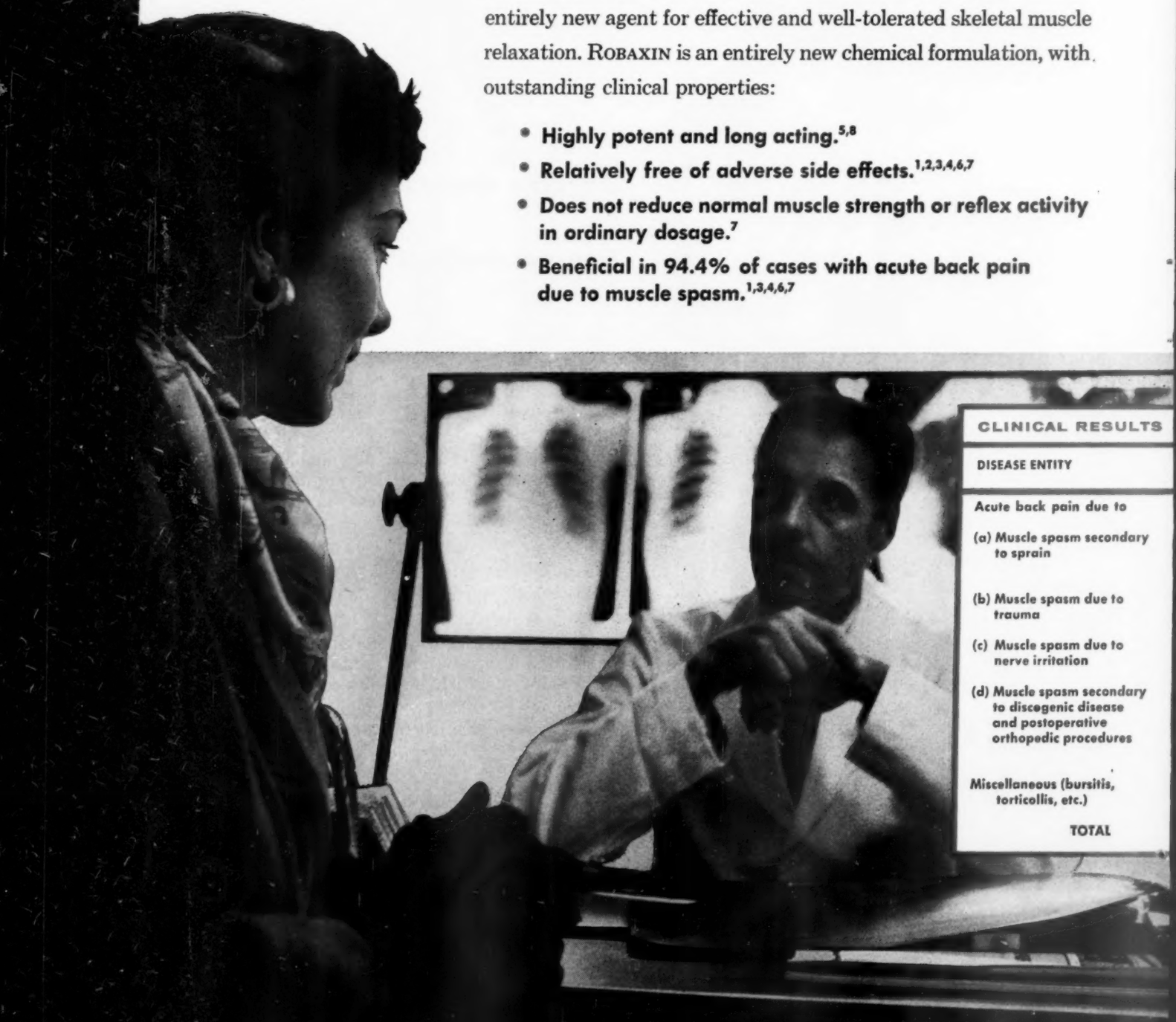
- **Highly potent and long acting.**[5,8]
- **Relatively free of adverse side effects.**[1,2,3,4,6,7]
- **Does not reduce normal muscle strength or reflex activity in ordinary dosage.**[7]
- **Beneficial in 94.4% of cases with acute back pain due to muscle spasm.**[1,3,4,6,7]



CLINICAL RESULTS
DISEASE ENTITY
Acute back pain due to
(a) Muscle spasm secondary to sprain
(b) Muscle spasm due to trauma
(c) Muscle spasm due to nerve irritation
(d) Muscle spasm secondary to discogenic disease and postoperative orthopedic procedures
Miscellaneous (bursitis, torticollis, etc.)
TOTAL

baxin®

(Methocarbamol Robins, U.S. Pat. No. 2770649)

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ROBAXIN is highly specific in its action on the internuncial neurons of the spinal cord – with inherently sustained repression of multisynaptic reflexes, but with no demonstrable effect on monosynaptic reflexes. It thus is useful in the control of skeletal muscle spasm, tremor and other manifestations of hyperactivity, as well as the pain incident to spasm, without impairing strength or normal neuromuscular function.

Beneficial in 94.4% of cases tested

When tested in 72 patients with acute back pain involving muscle spasm, ROBAXIN induced marked relief in 59, moderate relief in 6, and slight relief in 3 – or an over-all beneficial effect in 94.4%.[1,3,4,6,7] No side effects occurred in 64 of the patients, and only slight side effects in 8. In studies of 129 patients, moderate or negligible side effects occurred in only 6.2%.[1,2,3,4,6,7]

WITH ROBAXIN IN ACUTE BACK PAIN[1,3,4,6,7]

NO. OF CASES	DURATION OF TREATMENT	DOSE PER DAY (divided)	RESPONSE marked	mod.	slight	neg.	SIDE EFFECTS
18	2-42 days	3-6 Gm.	17	1	0	0	None, 16 Dizziness, 1 Slight nausea, 1
13	1-42 days	2-6 Gm.	8	1	3	1	None, 12 Nervousness, 1
5	4-240 days	2.25-6 Gm.	4	1	0	0	None, 5
30	2-28 days	1.5-9 Gm.	24	3	0	3	None, 25 Dizziness, 1 Lightheadedness, 2 Nausea, 2 *
6	3-60 days	4-8 Gm.	6	0	0	0	None, 6
72			59	6	3	4	*Relieved on reduction of dose

References: 1. Carpenter, E. B.: Publication pending. 2. Carter, C. H.: Personal communication. 3. Forsyth, H. F.: Publication pending. 4. Freund, J.: Personal communication. 5. Morgan, A. M., Truitt, E. B., Jr., and Little, J. M.: American Pharm. Assn. 46:374, 1957. 6. Nachman, H. M.: Personal communication. 7. O'Doherty, D.: Publication pending. 8. Truitt, E. B., Jr., and Little, J. M.: J. Pharm. & Exper. Therap. 119:161, 1957.

Indications – Acute back pain associated with: (a) muscle spasm secondary to sprain; (b) muscle spasm due to trauma; (c) muscle spasm due to nerve irritation; (d) muscle spasm secondary to discogenic disease and postoperative orthopedic procedures; and miscellaneous conditions, such as bursitis, fibrositis, torticollis, etc.

Dosage – *Adults:* Two tablets 4 times daily to 3 tablets every 4 hours. Total daily dosage: 4 to 9 Gm. in divided doses.

Precautions – There are no specific contraindications to Robaxin and untoward reactions are not to be anticipated. Minor side effects such as lightheadedness, dizziness, nausea may occur rarely in patients with unusual sensitivity to drugs, but disappear on reduction of dosage. When therapy is prolonged routine white blood cell counts should be made since some decrease was noted in 3 patients out of a group of 72 who had received the drug for periods of 30 days or longer.

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CONCLUSIONS:

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"it has fulfilled better than any previously tried medicaments all the qualifications" expected of a proctologic ointment[1]

"promotes smooth epithelization and healthy granulation tissue and accelerates healing."[1]

- Specially formulated for prolonged, unusual efficacy in relieving pain, itching, irritation and inflammation in non-surgical HEMORRHOIDS, PRURITUS ANI, FISSURES, PERIANAL DERMATITIS, PAPILLITIS, etc. Non-sensitizing.

Formula: **RECTAL** DESITIN OINTMENT contains high grade Norwegian cod liver oil, zinc oxide, lanolin, talcum, sodium lauryl sulfate, petrolatum q.s. Does **not** contain local anesthetics, narcotics, or "caine" drugs which might mask serious anorectal disorders.

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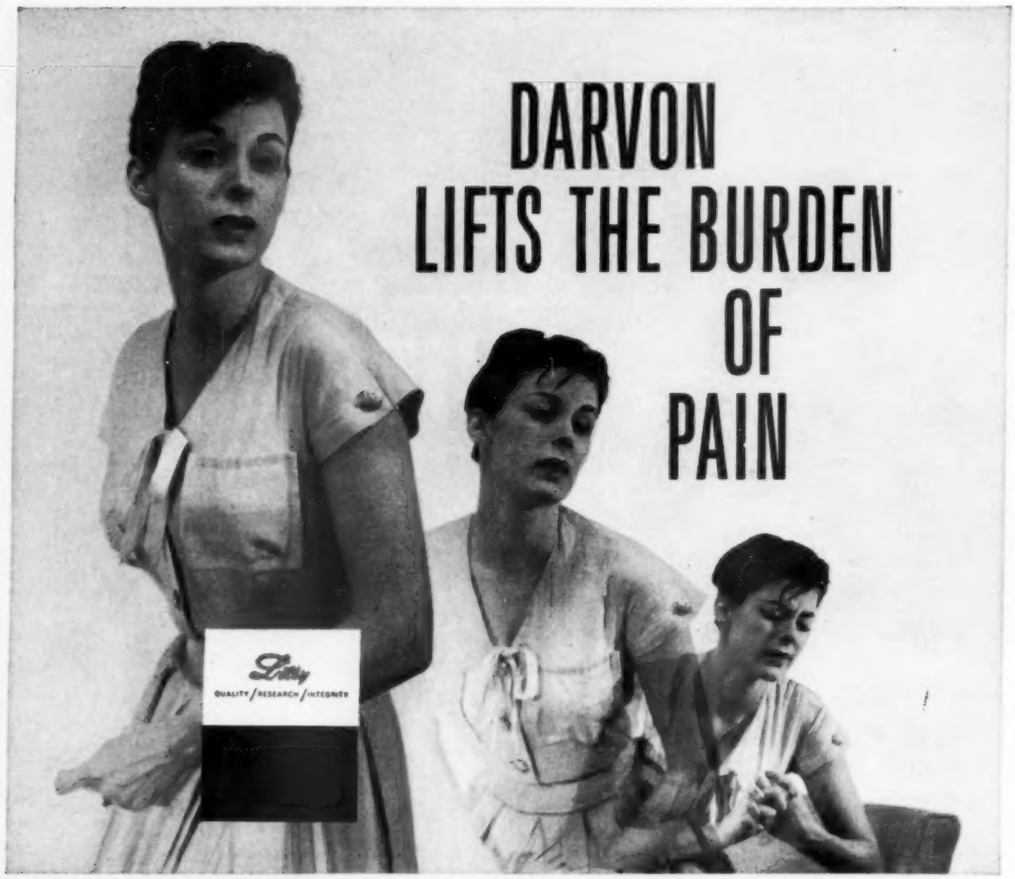
New **RECTAL** DESITIN OINTMENT is not to be confused with regular DESITIN OINTMENT

1. Spiesman, M. G. and Malow, L.: Amer. J. Proctology, June 1956.



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Each Pulvule 'Darvon Compound' provides:

'Darvon'	*32 mg.*
Acetophenetidin	*162 mg.*
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*'A.S.A. Compound' (Acetylsalicylic Acid and Acetophenetidin Compound, Lilly)

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The American Journal of Medicine

VOL. XXIV JANUARY, 1958 No. 1

Editorial

The Case against the Catheter

CATHETERIZATION of the urinary bladder is commonly regarded as a comparatively harmless procedure. Many physicians recommend that it be done as a matter of routine in female patients to obtain a satisfactory specimen for urinalysis. Indwelling catheters are placed in the bladder in order to secure specimens conveniently from patients who are unconscious or otherwise seriously ill, or to keep the bedding dry, or even to save a debilitated female patient the exertion of being placed on a bedpan. The belief is widely held that, with proper precautions, or that by using prophylactic chemotherapy, the risk of infection is negligible, or that at worst, simple cystitis is the only hazard. There is, however, reason to argue that, despite observation of the most careful technic, infection of the bladder not infrequently results from catheterization, and that this can lead to pyelonephritis, a disease of unquestioned gravity.

Part of the present uncertainty lies in the fact that there is no conclusive information about the pathway by which bacteria reach the kidney. The view is held by some that pyelonephritis is caused by bacteria which reach the kidneys by way of lymphatic or blood vessels. Others believe that infection usually travels upward to the kidneys from the bladder via the ureters. Evidence on this subject has been reviewed elsewhere in some detail [*1*]. Actually the exact path by which bacteria may travel from the bladder cavity to the kidney parenchyma is not an essential issue in the problem being discussed here, our main interest being to decide how common it is for pyelonephritis to develop as a sequel to infection which has begun in the bladder urine. From the standpoint of experiments in animals this pathway of infection has recently been shown to be possible: pyelonephritis having developed in rabbits following inoculation of colon bacilli into the bladder urine, without distention of the bladder or obstruction of the ureter [*2*].

What is the basis for suggesting that bacteria may be introduced into the bladder urine during a simple catheterization, despite the most careful antiseptic and aseptic technic? Certainly the catheter can be sterilized, and thorough cleansing of the surfaces around the urethral orifice should kill or wash away most of the bacteria residing there. The difficulty is that the urethral channel through which the catheter has to pass is not always sterile, nor can it readily be sterilized. Bacteria may therefore be transferred from the urethra into the bladder during passage of the catheter. This has been demonstrated to occur in a clinical experiment wherein pairs of urine specimens were cultured. One of each pair was obtained by direct aspiration from the bladder at laparotomy, the other was obtained by catheterization at the end of the operation. All twelve samples obtained by aspiration through the bladder wall were sterile, whereas four of twelve samples obtained from the same

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[2] BEESON, P. B., ROCHA, H. and GUZE, L. B. Experimental pyelonephritis: influence of localized injury in different parts of the kidney on susceptibility to hematogenous infection. *Tr. A. Am. Physicians*, 70: 120, 1957.

patients by catheterization gave positive cultures [3].

How often does cystitis develop following simple catheterization? Dysuria and pyuria may make little impression on the attending physician, so that their actual frequency may not be appreciated, but instances can be observed in any hospital, if looked for. Marple reported that manifestations of cystitis developed four times in a series of 100 catheterizations of female patients on a medical ward service [4], an incidence which seems in agreement with observations in our hospital. It is possible that *asymptomatic bacteriuria* follows catheterization with greater frequency, since urine in the bladder cavity provides a good environment for bacterial growth. Kass's studies have shown that significant numbers of bacteria may be found in the urine of patients who have no urinary symptoms [5]. David found that when he inoculated E. coli into dogs' bladder urines, signs of inflammation did not develop but that the organisms could be recovered by culture for as long as a month afterward [6]. Recently McDonald et al. have shown that there was a relationship between the finding of pyelonephritis at autopsy and the presence of significant numbers of bacteria in the bladder urine [7].

What is the likelihood of bladder infection when an indwelling catheter is employed? Kass found that in 95 per cent of patients so treated bacilluria developed within four days, in most of them within the first twenty-four hours, and that this could not be prevented by prophylactic use of antibiotics [5,8]. Furthermore, by means of an ingenious clinical experiment he and Schneiderman demonstrated that the pathway by which bacteria traveled to the bladder cavity was in the sheath of exudate which surrounds the catheter in the urethra [9]. Thus the patient with an indwelling catheter has an open communication between the bladder cavity and the outside of his body. The experiments of Weyrauch and Bassett demonstrated that motile bacteria could also ascend upward through the lumen of a catheter against the direction of flow [10]. Since this pathway of infection can be blocked by good aseptic technic, it seems that the real problem is the outside, not the inside, of the catheter.

Let us turn now to some items of clinical knowledge which suggest that the ascending pathway is of major importance in the total problem of kidney infection:

1. Acute, non-obstructive pyelonephritis is almost exclusively a disease of the female sex. The only reasonable explanation which has been offered for this is the fact that the female urethra is shorter and more liable to contamination by enteric bacteria.

2. Urinary tract infection is one of the principal threats to life of the patient with spinal cord injury or with poliomyelitis. The infection is often ascribed to "cord bladder," the assumption apparently being made that the malfunctioning bladder is more susceptible to infection because it is chronically overdistended. The fact is, however, that in such cases the first urine obtained by catheterization is nearly always free of bacteria and pus cells, and it is only after establishment of continuous vesical drainage that infection develops. Evidence of pyelonephritis may then make its appearance within a few weeks.

3. The observation has been made by several groups of workers that pyelonephritis is more likely to be a complication of obstructive disease of the urinary tract when the obstruction is in the bladder or urethra than when it is in the ureter [1]. Two explanations have been offered: vesicoureteral reflux due to chronic bladder distention, or greater frequency of catheterizations and cystoscopic treatments in the former group. Whichever is the correct explanation, both involve the assumption of spread of infection upward from bladder to kidneys.

4. Studies of the antibiotic sensitivities of

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bacterial strains recovered from patients have shown that resistant organisms are isolated much more frequently when the urine has become infected during treatment in a hospital than when infection was already present on admission. Particularly is this true of patients with indwelling catheters [*11*].

5. Cross infection has been shown to occur in urological wards, so that in patients being treated by catheterization there is a tendency for infections with the same bacterial strains to develop [*12,13*]. In an excellent study of this problem it was concluded that an important factor was transfer of bacteria from the catheter of one patient to that of another by the hands of the attending personnel [*14*]. This fits nicely with Kass and Schneiderman's experiment, already mentioned.

6. The nature of the bacteria encountered in infections which develop after some urologic procedure emphasizes the hazard of urethral instrumentation. When acute urinary infections develop in patients outside the hospital, E. coli is found to be the responsible organism in all but a few cases. When catheterization (and other forms of urethral instrumentation) have been employed, other bacteria, such as Proteus, Pseudomonas, Klebsiella and enterococcus make up a substantial proportion of the causative agents. The prediction can be made with confidence that whenever urine culture yields Proteus or Pseudomonas, investigation will show that the patient has previously been subjected to catheterization. While chemotherapy doubtless plays a part in this alteration in bacterial flora the fact remains that these resistant strains probably gained entry via the catheter.

It is not in the scope of this editorial to discuss the treatment or chemoprophylaxis of urinary tract infections. The inadequacy of our present methods of treatment [*8*] places a special obligation on us to avoid unnecessary risk of initiating urinary infection. Chemoprophylaxis may be of some value in preventing infection following a single catheterization, but has the limitation of being "blind" and not dependable to be effective against all possible contaminating micro-organisms. With regard to the indwelling catheter, where there is an open channel between bladder cavity and surface of the body, chemoprophylaxis could not be expected to, and indeed does not, do anything but eradicate susceptible organisms and favor the establishment of an infection more difficult to treat [*8*].

At times the catheter is indispensable for therapy, and there are many good indications for its use. Nevertheless the decision to use this instrument should be made with the knowledge that it involves risk of producing a serious disease which is often difficult to treat. The procedure does not seem warranted merely to obtain a clean specimen for routine urinalysis. It is not required even for bacteriologic study, since with proper precautions clean-voided specimens can be used for this purpose [*15*]. The indication should be especially clear when the decision is made to employ an indwelling catheter. Convenience of the attending doctors and nurses is surely not acceptable. Its use in the management of diabetic ketosis is especially risky since patients with diabetes are notably subject to urinary tract infections and to the severest forms of pyelonephritis.

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Clinical Studies

Characterization of the Anemia Associated with Chronic Renal Insufficiency*

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The anemia of chronic renal insufficiency is at present thought to be produced by a combination of erythroid depression and, in some instances, a hemolytic mechanism [*1–4*]. The present report, previously presented in abstract form [*5*], describes a series of investigations undertaken to define the anemia and its mechanism of production more precisely. The results indicate, as do those of other laboratories, that depression of erythropoiesis is the most important factor involved. In a minority of cases, however, blood loss from the gastrointestinal tract and/or an increased rate of red cell destruction are contributing factors. The hemolytic component occurred particularly in patients with chronic glomerulonephritis during the terminal stages of the disease and was shown to be caused by extracorpuscular factors. No information as to the nature of the substances responsible either for the erythroid depression or accelerated hemolysis was obtained in this study.

MATERIAL AND METHODS

Twenty-six anemic patients, eighteen to seventy-one years of age, with chronic renal insufficiency and azotemia form the basis of this study. The diagnosis in twelve patients was chronic glomerulonephritis; in eleven, hypertensive cardiovascular disease with nephrosclerosis; in one, hypertensive cardiovascular disease and chronic pyelonephritis; in one, arteriolar nephrosclerosis with acute pyelonephritis; and in one, polycystic disease of the kidneys. These individuals were either out-patients in the Washington University Clinics or were hospitalized in the Barnes Hospital as well as at the Jefferson Barracks Veterans Hospital. For the ten patients studied in greatest detail, brief clinical abstracts are given in the appendix: hematologic data are summarized in Tables I and II. It should be noted that 25 mg. non-protein nitrogen per 100 ml. plasma is the upper limit of normal in the Barnes Hospital laboratories because the protein is precipitated by zinc hydroxide [*6*] rather than the more usual phosphotungstic acid.

Osmotic fragility of erythrocytes was determined by the quantitative method of Suess et al. [*7*], using an Evelyn photoelectric colorimeter with modification as follows: (1) oxalated instead of heparinized blood was used; the mixture of sodium and potassium oxalate gave essentially the same fragility measurements as with heparin. (2) Beginning hemolysis was recorded as the concentration of saline solution in which at least 15 per cent hemolysis had occurred. (3) Twelve instead of twenty-four saline concentrations were routinely used: 0.0, 0.32, 0.38, 0.40, 0.44, 0.46, 0.48, 0.50, 0.52, 0.56, 0.60 and 0.85 gm. per cent. (4) A different clean mercury calibrated Sahli pipette was used for each blood aliquot introduced into the saline concentrations.

Mechanical fragility of erythrocytes (M. F.) was carried out with the technic of Shen, Castle and Fleming [*8*], each 0.5 cc. blood sample being rotated for one and a half hours, in contact with ten 1 mm. glass beads, at 32 r.p.m. with a wheel diameter of 8 cm. The M.F. is expressed as per cent hemolysis. Normal values are given in Table IV. Each M.F. value recorded is the average of duplicate determinations obtained at a time when the M.F. of a duplicate control was within the range of normal.

Direct Coombs' test [*9,10*] was performed with a 5 per cent suspension of patients' red cells which had been washed three times with 0.85 per cent saline solution. Two drops of the red cell suspension were added to 2 drops of anti-human globulin serum in a 10 by 75

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TABLE I

CLINICAL DATA ON TEN PATIENTS WITH THE ANEMIA OF RENAL INSUFFICIENCY

Case No.	Patient	Age (yr.)	Diagnosis	Red Blood Cells* (millions per cu. mm.)	Hemoglobin* (gm./100 cc.)	Packed Red Cell Volume* (%)	Reticulocytes† (highest %)
1	L. N.	28	Chronic glomerular nephritis	1.77	5.6	16.5	11
2	C. L.	25	Nephrosclerosis, acute pyelonephritis	2.27	5.8	17	11.4
3	M. W.	34	Chronic glomerular nephritis	1.48	5.1	14.5	13.2
4	H. B.	30	Chronic glomerular nephritis	2.08	6.7	19	22.5
5	B. B.	25	Chronic glomerular nephritis	2.06	6.7	19	5
6	J. S.	70	Chronic glomerular nephritis, arteriolar nephrosclerosis	2.30	7.7	23.5	6.6
7	W. T.	50	Chronic glomerular nephritis	3.51	8.4	27	7.0
8	S. M.	61	HCVD, nephrosclerosis	2.24	6.4	21	6.7
9	G. H.	53	HCVD, pyelonephritis	2.16	6.5	21.5	5.6
10	P. L.	18	Chronic glomerular nephritis	2.47	7.3	22	7.8

* Lowest values encountered during observation period.
† Highest value during observation period.

TABLE II

LABORATORY DATA ON 10 PATIENTS WITH THE ANEMIA OF RENAL INSUFFICIENCY

Case No.	Patient	Reticulocytes (average %)	Mean Corpuscular Volume* (cu. microns)	Mean Corpuscular Hemoglobin (μμg.)	Mean Corpuscular Hemoglobin Concentration (%)	Plasma Nonprotein Nitrogen (mg. % range)	Serum Iron (μg. % range)	Free Erythrocyte Protoporphyrin (μg. % range)	Red Blood Cell Half-life (in days)
1	L. N.	7.7	89	32	35	(B.U.N.) 48–192	.....	.....	21
2	C. L.	8.6	91	30	33	(B.U.N.) 84–162	46–58	139	18
3	M. W.	8.2	93	32	34	140–278	35–94	186–249	35
4	H. B.	8.4	86	30	35	78–420	19–100	20–60	44
5	B. B.	2.4	85	30	36	80–342	26–100	15–36	38
6	J. S.	3.4	100	34	34	50–90	67–127	10–25	52
7	W. T.	4.5	81	27	34	50–94	40–68	38–132	68
8	S. M.	3.6	95	31	32	55–95	51–86	24–29	68
9	G. H.	3.8	96	31	34	95–177	43	45	55
10	P. L.	4.3	90	29	33	(B.U.N.) 43–65	45	.....	38

* Corpuscular contents represent average of five or more values.

mm. test tube and incubated at room temperature for five minutes. After being centrifuged for five minutes (1,500 r.p.m.) the tube was tilted with a to-and-fro motion for maximum mixing of the dislodged red cell mass; agglutination was read both macroscopically and microscopically. Control tests were made in each case with Rh-positive cells to which anti-Rh blocking antibodies had been adsorbed *in vitro*.

The Coombs' test using trypsinized red cells [*11*] was carried out in approximately one-third of the patients using an 0.1 per cent solution of trypsin in 0.85 per cent saline solution for the second washing of the 5 per cent red cell suspension. The red cells were incubated at 37°C. for twenty minutes in this suspension; the Coombs' test was then made.

Free erythrocyte protoporphyrin values were determined by the spectrophotometric micromethod of Grinstein and Wintrobe [*12*]. The serum iron was determined by the thiocyanate method employed in this laboratory [*13*]. The utilization of *intravenously administered radioiron* for hemoglobin formation was measured by a method reported in detail previously [*14*]. The radioactive iron was injected intravenously as ferrous ascorbate in amounts of 8 to 12 mg. expressed as elemental iron. Preparations of higher specific activity were not available at the time these tests were performed; therefore, larger amounts of iron were given than in more recent studies. Following the injection of the radioactive iron, radioactivity measurements were made at three-day intervals on venous blood samples and the result was expressed as the per cent utilization of the injected dose. Fecal excretion of urobilinogen was determined in four-day collections. The method of Watson et al. was used without modification [*15*]. Bilirubin determinations were made by the method of Ducci and Watson [*16*].

For peripheral blood counts, U. S. Bureau of Standards calibrated pipettes and counting chambers were used. Hemoglobin values were determined by the Evelyn oxyhemoglobin method, using mercury standardized Sahli pipettes. Hematocrits were estimated in Wintrobe tubes; the final reading was made after centrifugation for thirty minutes at 3,000 r.p.m. Reticulocyte preparations were stained supravitally with brilliant cresyl blue [*17*]. Normal values in our hands ranged from 0.5 per cent to 2.0 per cent.

Survival of transfused red cells was followed by a modification of the Ashby technic for differential agglutination [*18,19*]. In this method 1 cc. of oxalated venous blood was diluted to 100 cc.; the resulting cell suspension was mixed with equal volumes of the appropriate potent anti A, anti B or anti M serum, allowed to stand a few minutes, then centrifuged slowly (1,000 r.p.m.) for ten minutes. The tubes were tapped forty times and counted in duplicate by two observers using Spencer bright-line hemocytometers. With the antiserums used, from 6,000 to 56,600/cu. mm. unagglutinated cells were present in the recipients' blood prior to transfusions. The pretransfusion unagglutinated cell count was subtracted from the total unagglutinated cell count post-transfusion to determine the number of surviving transfused red cells. These determinations were made at weekly intervals, with more frequent counts during the first week following transfusion.

TABLE III
SERUM IRON AND FREE ERYTHROCYTE PROTOPORPHYRIN (E.P.) IN ANEMIA OF RENAL INSUFFICIENCY

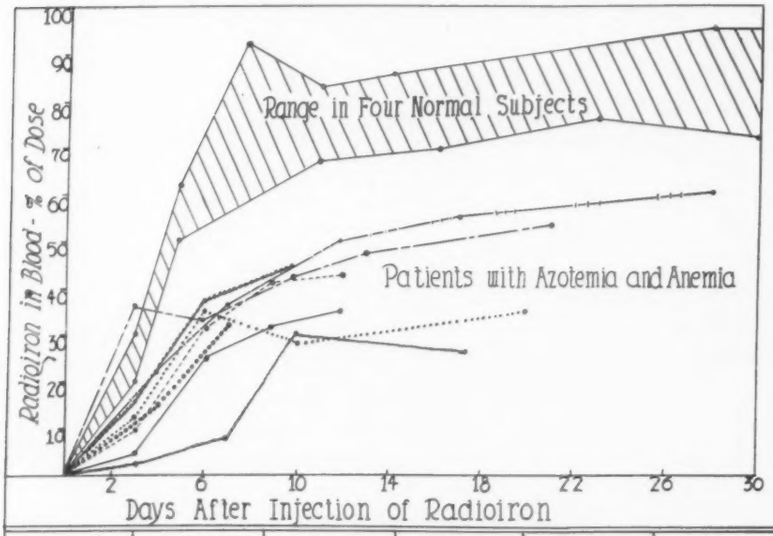
No.	Patient	*Average E.P.** Male	*Average E.P.** Female	Serum Iron (μg./100 cc.)
0	Normal	13–55	16–70	50–180
1	B. M.	.....	46	38
2	J. S.	19	.....	101
3	H. J.	50	.....	104
4	H. B.	28	.....	44
5	F. M.	.....	54	102
6	S. L.	.....	111	125
7	M. G.	.....	13	42
8	B. B.	116	.....	99
9	G. N.	.....	43	79
10	W. T.	104	.....	45
11	W. W.	113	.....	89
12	E. H.	321	.....	62
13	M. W.	223	.....	59
14	G. M.	27	.....	89
15	L. M.	56	.....	91
16	S. M.	27	.....	69
17	E. H.	47	.....	84
18	C. R.	108	.....	78
19	C. L.	139	.....	52
20	R. N.	58	.....	65
21	G. H.	45	.....	43

* Values are in gamma/100 ml. of packed red cells.

RESULTS

Morphologic and Chemical Characteristics of the Anemia of Uremia. Corpuscular constants: The anemia in these twenty-six patients with renal insufficiency was uniformly normochromic and most often normocytic (Table II); in three instances, the mean corpuscular volume was consistently above 100 cu. microns. No mean corpuscular volume in any patient was less than 81 cu. microns.

Serum iron: Serum iron determinations were made in twenty-one patients; in some, serial results were obtained. The serum iron varied between 38 to 125 μg. per cent. (Table III.) Normal values in this laboratory are 50 to 180 μg.



Legend	Subject	Age	R.B.C. millions	MCHC %	NPN mg.%
———	B.B. #1	25	2.27	36	83
.....	M.W.	34	2.51	35	150
— — —	J.S.	70	3.08	36	71
——+——	H.J.	48	4.19	35	77
·····+·····	S.M.	61	2.35	32	50
·····+·····	G.H.	53	2.16	30	124
·—·—·—·	B.B. #2	25	3.01	38	90
———	M.K.	68	2.89	33	80

FIG. 1. This figure illustrates the poor utilization of radioactive iron for hemoglobin production in patients with azotemia and anemia, evidence that erythropoiesis was depressed in the presence of renal insufficiency.

per cent. Values below 50 μg. per cent were obtained in five patients. Thus in these twenty-one patients the serum iron value was either within the normal range or decreased.

Free erythrocyte protoporphyrin (E.P.): Eighty-nine determinations of free erythrocyte protoporphyrin were made in twenty-one patients. The average values are listed in Table III. Significantly elevated values were found in eight of the twenty-one patients (38 per cent).

The results of these investigations showed that there was probably no consistent relationship between the E.P. and serum iron. (Table III.) These results could not be correlated with the type of renal disease, degree of azotemia, severity of anemia, or with the presence or absence of a hemolytic component. Further studies were therefore initiated to assess the factors of depressed erythropoiesis, hemolysis and external blood loss as causes of the anemia in these patients. The results of these studies will be presented in the following sections.

Utilization of Intravenous Radioiron. The utilization of radioactive iron was determined eight times in seven patients as a measure of erythropoietic activity. (Fig. 1.) A significantly decreased utilization, as compared to normal controls, was found in each subject. The other data tabulated in Figure 1 were obtained just prior to the injection of the radioactive iron. Six of the utilization determinations were made immediately prior to the transfusion studies which are reported in the section on hemolytic studies. There was no evidence of increased destruction of red cells in five measurements in four patients (Fig. 1, B. B. No. 1, B. B. No. 2, J. S., S. M., G. H.). The decreased utilization of intravenous radioiron in these patients therefore most likely represented depressed erythropoiesis. In one patient (M. W.) (Fig. 1) increased destruction of red cells was demonstrated and the poor utilization of radioiron in this patient cannot be ascribed entirely to a depression of erythropoiesis. The decreased radioactive iron utilization in the presence of hemolytic disease has been attributed to the destruction of erythrocytes which contain radioactive iron shortly after their delivery into the peripheral blood [*20*]. It has also been sug-

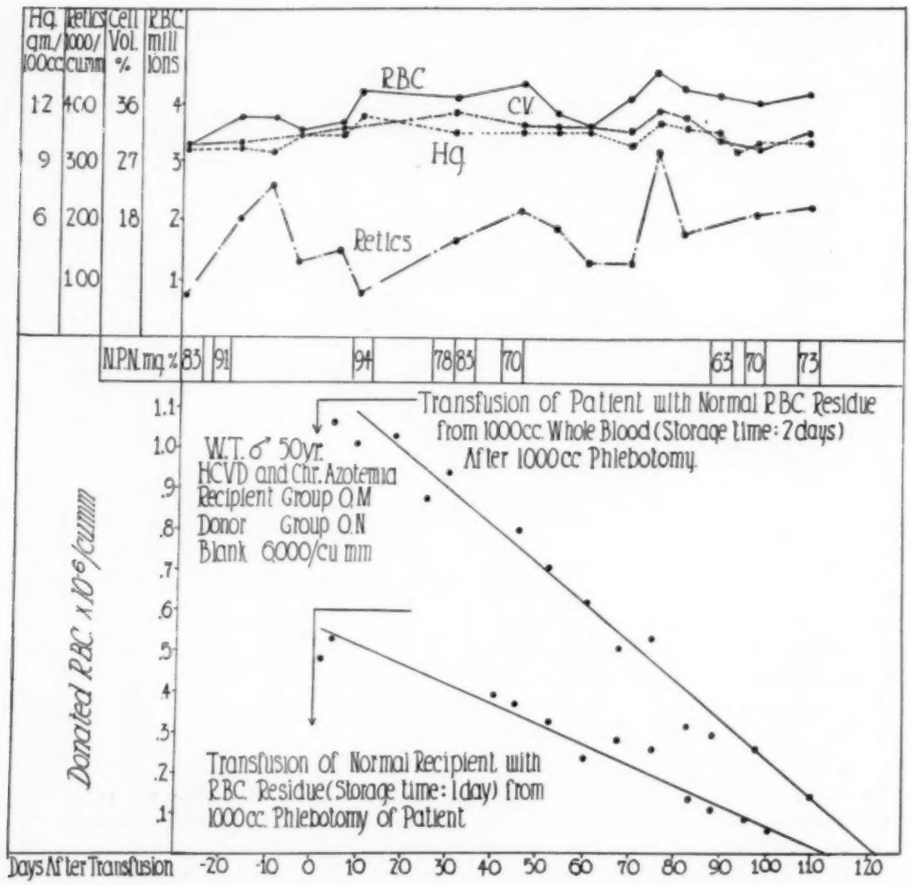


FIG. 2. Patient W. T. given transfusion of normal erythrocytes. These cells survived a normal length of time (upper curve), as did the patient's own cells (lower curve) in a normal recipient. During the observation period the peripheral blood values remained quite stable.

gested that in the presence of hemolysis the injected radioiron is immediately deposited in inactive tissue stores [*1*].

Hemolytic Factors. Although the information gathered by the radioactive iron studies pointed to depressed erythropoiesis as the main cause of the anemia associated with chronic renal disease it was noted that some patients became rapidly anemic in the absence of detectible bleeding. An investigation of possible hemolytic factors as a cause of the anemia was carried out by direct and indirect methods and will be reported in this section.

Reticulocytosis: A slight to moderate reticulocytosis was found in all twenty-six patients at one time or another. In most patients there was only an occasional elevation; in others, the number of reticulocytes was consistently increased in number. (Tables I and II.) It is apparent that patients one through four (Table I) had a more striking reticulocytosis than the others.

Red cell survival studies: Hemolytic anemias may be caused by either intra- or extracorpuscular mechanisms. Possible intracorpuscular defects were studied in these patients by transfusion of the patients' cells into normal subjects. From five patients 800 to 1,000 cc. of blood was obtained; the red cells were allowed to settle and were transfused following the removal of the plasma. The survival of these cells was followed by the technic of differential agglutination.

The patient's cells in every case showed a normal survival rate in the circulation of a normal individual. The lowermost curve in Figures 2 and 3 represents the survival of the patients' cells which were transfused into normal subjects in two cases and were typical of the other results. It should be noted that in C. L. (Fig. 3) the patient's own cells had a normal survival in a normal individual at a time when the transfused cells were being rapidly destroyed in the patient

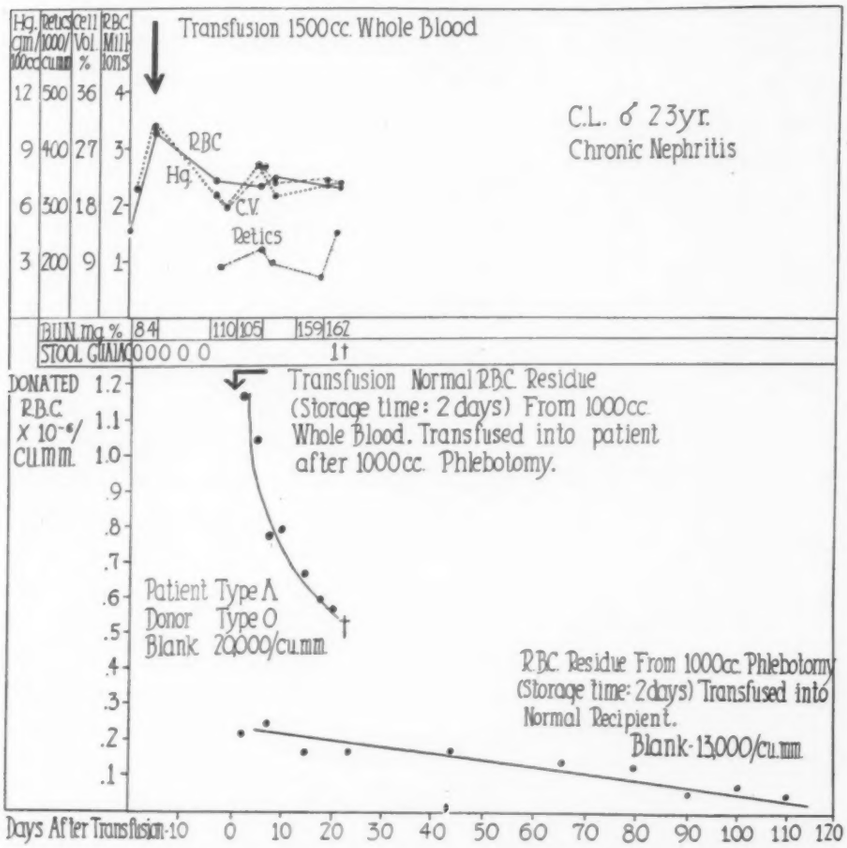


Fig. 3. C. L., a patient with chronic glomerulonephritis, progressive azotemia and rapidly increasing anemia. Transfused red cells (upper curve) from a normal donor were apparently disappearing at an accelerated rate when the patient died twenty days after the transfusion. The patient's red cells from a 1,000 cc. phlebotomy were transfused (lower curve) into a normal recipient and survived the expected normal time.

(upper curve, Figure 3). This result clearly demonstrated an extracorpuscular hemolytic component.

Since no intracorpuscular defects could be demonstrated, possible extracorpuscular factors were measured by following the survival of red cells obtained from normal subjects transfused into the patients. Twelve patients were given transfusions in this manner and it was possible to follow up ten patients to the completion of their red cell survival studies. In these ten, thirteen studies were completed.

In four of these ten patients the transfused cells survived normally (Table II, J. S., W. T., S. M., G. H.). Figure 2 is a graphical presentation of the data on one of these four patients; data on two others are included in Figure 5.

These four patients had a similar clinical course in that their peripheral blood values were quite stable, both in the months preceding the transfusion studies and during the succeeding months. Three of these patients (J. S., W. T., S. M.) were followed up to the time of their death and no sudden or marked fall in their peripheral blood values occurred. In one patient (J. S.) a second Ashby curve was completed several days before death and a normal red cell survival was again demonstrated. (Fig. 5.)

In contrast, five patients demonstrated an element of increased hemolysis by an increased rate of disappearance of transfused normal cells (L. N., C. L., M. W., B. B., P. L., Table II). The survival times of the transfused normal cells in these subjects were shortened to thirty-six to seventy-six days instead of the expected ninety to 130 days. Figures 3 and 4 present the individual data on two patients. Figure 5 shows the distinct difference between the normal survival in some patients and the shortened survival in others. In one patient (B. B., Table II), the trans-

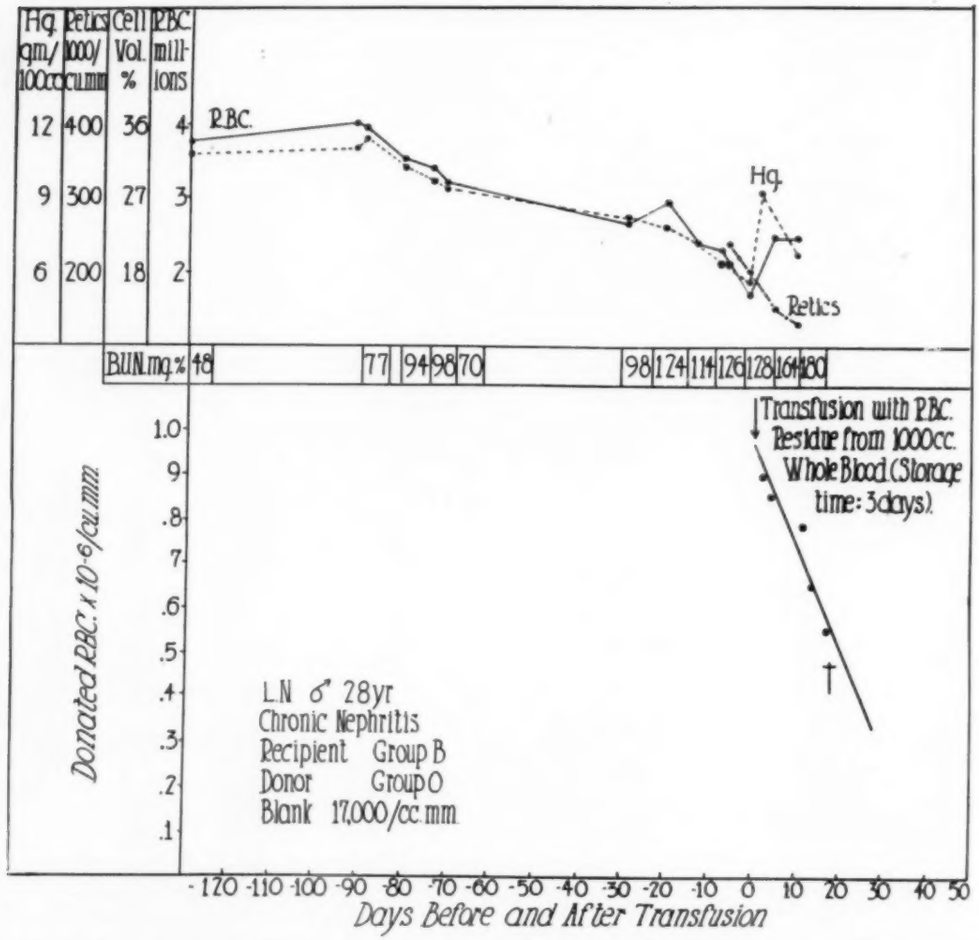

Hg. gm./100cc.
Retics 1000/cu.mm
Cell Vol. %
R.B.C. millions
12 400 36 4
9 300 27 3
6 200 18 2
R.B.C.
Hg.
Retics
B.U.N. mg.%
48 77 94 98 70 98 124 114 126 128 164 180
Transfusion with R.B.C. Residue from 1000cc. Whole Blood (Storage time: 3 days)
Donated R.B.C. x 10-6/cu.mm
1.0 .9 .8 .7 .6 .5 .4 .3 .2 .1
L.N ♂ 28yr
Chronic Nephritis
Recipient Group B
Donor Group O
Blank 17,000/cc.mm
-120 -110 -100 -90 -80 -70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50
Days Before and After Transfusion

FIG. 4. L. N., a patient with chronic glomerulonephritis progressive azotemia and rapidly increasing anemia, was transfused with red cells from a normal donor. These cells were apparently disappearing at a faster than normal rate when the patient died eighteen days after the transfusion.

fusion of normal red cells during a period when he was becoming progressively anemic showed a T/2 survival of thirty-eight days. Five months later, at a time when his peripheral blood values were quite stable, a repeat study showed normal cells to have a T/2 survival of fifty days. The mild extracorpuscular hemolytic factor present at the time of the first transfusion was evidently not active at the time of the subsequent transfusion.

All five patients in whom an extracorpuscular hemolytic factor was demonstrated differed clinically from the previously mentioned group with normal survival times. The survival times in these five patients were obtained during the terminal phase of their illness; four patients died within sixty-five days after transfusion studies were initiated. They were becoming rapidly anemic. The plasma non-protein nitrogen levels at the initiation of the transfusion studies were above 91 mg. per cent, with one exception (P. L.), in contrast to levels below 83 mg. per cent for the non-hemolytic group. The patients ranged in age from twenty-five to thirty-five years, considerably younger than the former group whose ages varied from fifty to seventy years.

One patient (H. B., Table II) resembled the hemolytic group in his terminal precipitous anemia and pronounced azotemia, yet the survival of normal cells showed a T/2 of forty-four days and was therefore equivocal.

These studies demonstrated that an extracorpuscular hemolytic mechanism is at times present in patients with chronic renal insufficiency.

Coombs' test: The Coombs' test was negative in all twenty-six patients in this series. The

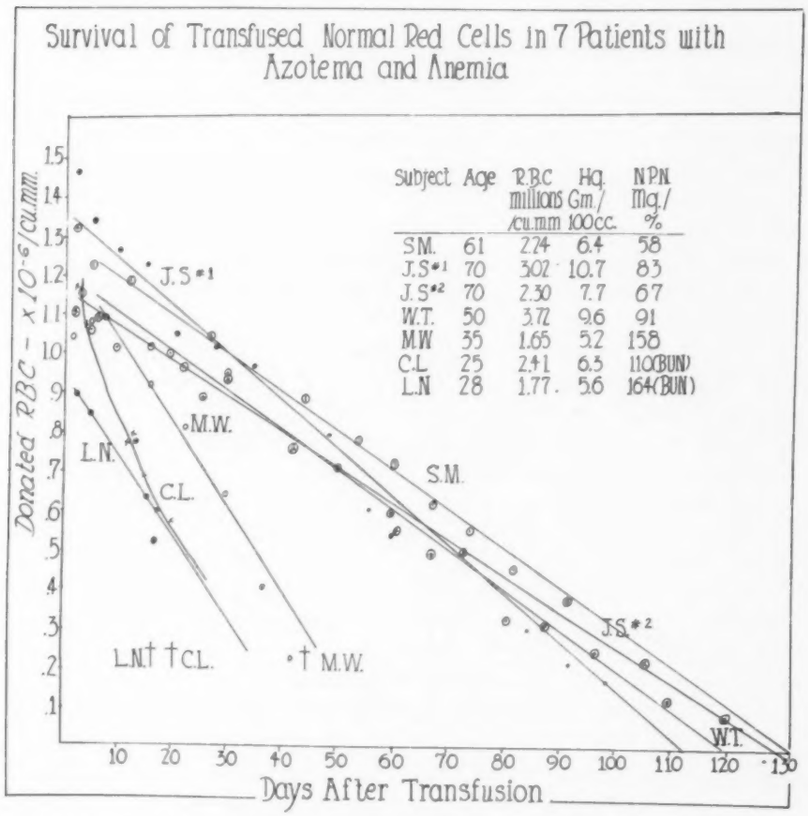

Survival of Transfused Normal Red Cells in 7 Patients with Azotemia and Anemia
Donated RBC - x 10⁻⁶/cu.mm.
Days After Transfusion
Subject Age R.B.C. millions /cu.mm Hg. Gm./ 100cc. N.P.N. Mg./ %
S.M. 61 2.24 6.4 58
J.S.#1 70 3.02 10.7 83
J.S.#2 70 2.30 7.7 67
W.T. 50 3.72 9.6 91
M.W. 35 1.65 5.2 158
C.L. 25 2.41 6.5 110(BUN)
L.N. 28 1.77 5.6 164(BUN)
J.S.#1
M.W.
L.N.
C.L.
S.M.
J.S.#2
L.N. † C.L.
† M.W.
W.T.

FIG. 5. The upper four curves (J. S. No. 1, J. S. No. 2, S. M., W. T.) illustrate the normal survival of transfused red cells in patients with azotemia and anemia. The three lower curves (L. N., M. W. and C. L.) illustrate the presence of an extracorpuscular factor shortening the survival of transfused red cells. The plasma nonprotein nitrogen and blood urea nitrogen values listed are those obtained at the initiation of each transfusion.

Coombs' test using trypinized cells was negative in five patients.

Osmotic and mechanical fragilities: Twenty-two determinations of the osmotic fragility were made on the red blood cells of nine patients. (Table IV.) Beginning hemolysis in a concentration of saline above 0.48 per cent was found in only three instances. In all three of these patients (L. N., M. W., B. B.) an extracorpuscular hemolytic factor had been demonstrated.

The *in vitro* mechanical fragility of the patients' erythrocytes was determined on twenty-nine occasions in seven patients. (Table IV.) The results, in general, were within the normal range of values. As a consequence, this test was of no value in distinguishing those patients who had an extracorpuscular hemolytic component.

Serum bilirubin: One-minute and total bilirubin determinations were made at least once in every patient. Values within the normal limits were obtained in all determinations. Serial determinations were made in patients exhibiting a hemolytic component but the values remained within the normal range.

Fecal excretion of urobilinogen and hemolytic indices: Fecal urobilinogen determinations were made and hemolytic indices were calculated in five patients. (Table V.) The fecal urobilinogen value was within normal limits in all five patients. When the hemolytic index was calculated, however, only two values were within the normal range. Normal cells transfused into these two patients had a normal survival time. Elevated hemolytic indices of 23, 32 and 40 were obtained in three patients, all of whom had a hemolytic component demonstrated by the shortened survival of normal cells.

Blood Loss as a Contributing Factor. In two of our patients significant bleeding tendencies developed while they were under study. In one (B. B., Table I), during the last sixty days of his illness, there was frequent epistaxis, and gross

TABLE IV

SUMMARY OF FRAGILITY STUDIES, COOMBS' TESTS AND RED CELL SURVIVAL IN ANEMIA OF RENAL INSUFFICIENCY

Patient	Osmotic Fragility			Mechanical Fragility				Direct Coombs' Test		Average‡ Red Blood Cell Half-life (in days)
	Range*	Average	No. Determinations	Range†	Mean	Average	No. Determinations	Patient's Red Blood Cells	Trypsinized Red Blood Cells	
Normal	.40–.48	...	..	1.8–5.9	3.9	4.2	35	0	0	45–65
L. N.	.52–.56§	.55	3	4.0–6.2§	5.1	5.0	5	Negative	Negative	21
C. L.	.40	.40	2	5.2–5.5	5.3	5.4	5	Negative	Negative	18
M. W.	.52	.52	1	.....	...	...	..	Negative	.....	35
H. B.	.44	.44	1	.....	...	...	..	Negative	.....	44
B. B.	.44–.52	.48	7	4.7–6.3	5.5	5.0	4	Negative	.....	38
J. S.	.46–.48	.47	2	3.8–4.8	4.3	4.3	5	Negative	Negative	52
W. T.	.40	...	1	3.8	...	...	1	Negative	.....	68
S. M.	.40–.48	.43	3	4.1–5.5	4.8	4.5	7	Negative	.....	68
G. H.	.44	.44	2	4.8–5.4	5.1	5.1	2	Negative	.....	55
P. L.	.....	...	..	.....	...	...	..	Negative	Negative	38

* Values refer to per cent saline concentration in which beginning hemolysis occurred.
† Values are expressed as per cent hemolysis.
‡ Values represent time when half the normal cells transfused into patient had disappeared.
§ Range for serial determinations.

TABLE V

FECAL EXCRETION OF UROBILINOGEN IN ANEMIA OF RENAL INSUFFICIENCY

Patient	Weight (Kg.)	Hemoglobin (gm./100 cc.)	Average* Daily Fecal Weight (gm.)	Urobilinogen Excretion Per Day (mg.)	Hemolytic† Index	% Above Expected Maximum	Average Half-life of Transfused Normal Red Blood Cells
M. W.	51	5.1	236	90	40	100	35
H. B.	52	7.4	295	99	32	60	44
B. B.	54	8.0	87	80	23	15	38
J. S.	57.6	12.3	132	52	9.2	0	52
W. T.	77.4	10.5	219	67	10.3	0	68

* Figures refer to average daily stool weight based on a four-day collection period.
† Normal range, 11–20.8.

gastrointestinal blood loss occurred almost continually. During this period the apparent survival time of transfused normal cells was sixty days but some of the shortening most likely represented the loss of some of the transfused cells through hemorrhage. One other patient manifested gross gastrointestinal bleeding and epistaxis soon after the transfusion of normal cells. His anemia rapidly became more severe and he died thirty days later.

In none of the other patients did significant blood loss develop during the period of study. Frequent guaiac tests were made on stool specimens and the patients were closely questioned about other sites of hemorrhage. Only three patients had histories of infrequent epistaxis in the months preceding the study.

COMMENTS

An anemia eventually occurs in almost every patient with uremia regardless of the nature of

the underlying renal lesion [*21,22*]. However, the anemia may not be present for many years despite chronic renal insufficiency [*23*]. In a large series of cases, Callen and Limarzi characterized the anemia as normochromic and normocytic [*24*]. Our results confirmed this finding.

In certain anemias rather consistent changes in serum iron levels are seen. Low values can be expected: (1) in iron deficiency, (2) with rapid hemoglobin formation, and (3) in the anemia of infection, in which iron is diverted rapidly to tissue stores [*25*]. In contrast, elevated serum iron values are noted in (1) anemias in which lack of iron is not a factor yet erythropoiesis is depressed (i.e., pernicious anemia and aplastic anemia), and (2) hemolytic anemia in which there is an increased breakdown of hemoglobin and consequent excessive liberation of iron. It is somewhat surprising that elevated values were not found in this series of patients, probably because multiple factors were playing a role. Joske et al. [2] in their study also found normal serum iron values in patients with uremia.

Elevated values of free erythrocyte protoporphyrin have been found in the anemias of iron deficiency, heavy metal intoxication, chronic infection, leukemia, Hodgkin's disease and multiple myeloma as well as in the hemolytic and refractory anemias [*26*]. No consistent pattern was found in this series of patients although elevated values were obtained in eight of twenty-one patients. Cartwright has reported elevated erythrocyte protoporphyrin values in six of seven patients with anemia and azotemia [*27*]. However, no consistent pattern has developed such as is seen in the anemia of infection.

Erythroid depression has been regarded as the most important cause for the anemia associated with chronic renal insufficiency. Two general methods, bone marrow examination and utilization of radioactive iron, have been used to assess erythropoietic activity in chronic renal disease.

Leitner [*28*], Dameshek [*29*], Nordenson [*30*] and others [*31–33*] all have described a selective decrease in the cellularity of the bone marrow erythrocyte precursors, myeloid and megakaryocytic elements not participating in the reaction. Some observers have noted an increasing aplasia of erythropoietic tissue with progressive renal failure [*31*]. In some of the cases reported an increased number of normoblasts was found [*34,35*]. Callen and Limarzi [*24*], in a study of the bone marrow in renal disease, found a hypercellular marrow in 80 per cent of forty-four patients and no cases of aplasia. The myeloid-erythroid ratio increased moderately as the plasma non-protein nitrogen rose. They stated, "Definite evidence of quantitative and qualitative hypoplasia of the erythroid tissue in renal disease was observed only when the non-protein nitrogen level was over 150 mg." They believed that the hemopoietic state of the marrow had no prognostic significance.

More precise information regarding erythropoietic activity has been obtained from iron utilization studies. Finch et al. showed decreased iron utilization in five patients with uremia [*1*]. Joske, McAlister and Prankerd, who recently studied fifteen patients with chronic renal disease, also found a decreased utilization of iron [*2*]. Since similar results were found in the present study, it seems quite clear that there is a decreased ability of the bone marrow to utilize radioactive iron in patients with uremia and that this represents the fundamental cause of the anemia. It is of interest that Markson and Rennie [*36*], using tissue culture technics, found that serum from uremic patients caused a partial inhibition of maturation of immature erythroid cells.

It is not known whether the depressed erythropoiesis in uremia results from the toxic action of a retention product on red cell production or from the lack of a specific erythroid stimulating factor. Germane to this problem are the recent investigations of Jacobson et al. [*37*]. They believe that erythropoietin may be produced in the kidney since it could not be demonstrated in rats following bilateral nephrectomy. Many other organs had been removed without suppression of erythropoietin formation.

While depressed erythropoiesis remains the cause of the anemia of nitrogen retention, this study demonstrated that an extracorpuscular hemolytic factor is sometimes present. Emerson and Burrows in 1949 also found excessive hemolytic activity to be a contributing factor [*4*]. Since the publication of these results, they have been confirmed in other laboratories. Chaplin and Mollison in 1953, using normal cells for transfusion, found a diminished red cell lifespan in five of six patients in the terminal stage of nephritis [*3*]. They also found a normal red cell lifespan in three patients whose disease was relatively stable. Sutherland et al., using autotransfused cells tagged with Cr^{51}, found significantly lowered survival times in nine of the eleven patients they studied [*38*]. Recently,

Joske, McAlister and Prankerd found a distinctly shortened red cell lifespan of normal cells in four of fifteen patients with chronic renal disease [*2*]. These studies have all definitely demonstrated that an extracorpuscular hemolytic mechanism is at times present.

In this study and in Joske's study no intracorpuscular defect was demonstrated by transfusion of red cells from the patients into normal recipients. However, Sutherland and his group did observe an intracorpuscular defect in two of their cases [*38*]. Furthermore, they found that red cells from dogs made uremic by bilateral nephrectomy had a shortened survival time when transfused into normal dogs. Thus it may be, although not demonstrated in the present study, that an intracorpuscular defect may at times be present.

While a hemolytic mechanism has been demonstrated, the cause is still unknown. The Coombs' test in the present and in Joske's study was consistently negative so an autoimmune mechanism of destruction has not been found. The abnormal osmotic fragility noted in some of the patients reported in this paper was probably correlated with the presence of the hemolytic factor, as was the demonstration of an elevated hemolytic index in some patients. It is also of interest that the serum bilirubin values remained normal even when increased hemolysis was demonstrated.

A definite hemorrhagic diathesis sometimes occurs in uremia. However, blood loss is probably of incidental importance in the pathogenesis of the anemia of uremia. Only two of twenty-six patients had significant external loss of blood.

SUMMARY

The anemia occurring with chronic renal insufficiency was studied in twenty-six patients. From this study it is possible to draw the following conclusions:

1. The anemia is usually normocytic and normochromic although an occasional patient has a consistently macrocytic mean corpuscular volume.

2. Serum iron and free erythrocyte protoporphyrin values follow no consistent pattern.

3. There is an invariable depression of erythropoiesis, demonstrated in these patients by poor utilization of radioiron. This constitutes the prominent mechanism responsible for the anemia.

4. Erythrocytes formed under conditions of severe renal insufficiency and azotemia are normal as measured by their ability to survive normally in healthy recipients.

5. Occasionally the anemia becomes rapidly progressive in the absence of detectible blood loss. At these times an unidentified extracorpuscular hemolytic factor is present and presumably is responsible for the increased breakdown of red cells. Thus the anemia must at times be due to a combined disorder of depressed erythropoiesis and increased red cell destruction.

6. Blood loss infrequently accounts for a significant part of the anemia, but in individual cases may be a third responsible factor.

APPENDIX

CASE 1. L. N., a twenty-eight year old white man, was entirely well until December, 1948, when malaise developed with migrating joint pains accompanying swelling, tenderness and redness involving alternatively both knees and the right ankle. A diagnosis of acute rheumatic fever was made. Several weeks later, generalized swelling of the face, eyes and extremities appeared. At this time he was told he was losing protein and red cells in his urine and that he had an elevated blood pressure. The diagnosis was acute glomerulonephritis. Edema disappeared after several more weeks but considerable malaise remained. No joint symptoms followed the onset of his edema.

The past history was negative for any antecedent joint or renal involvement.

In December, 1949, urinalysis consistently showed 3 to 4 plus proteinuria, numerous red corpuscles, a few white blood cells and a few hyaline and granular casts. During the first nine months of his illness his red cell count varied between 3.7 and 2.7 million per cu. mm., blood urea nitrogen between 39 and 60 mg. per cent; his blood pressure was consistently near 180/110 mm. Hg.

His last three months were characterized by gradually increasing blood urea nitrogen, progressive anemia, and a steady downhill course until his death, twelve months after the onset of illness. Laboratory data obtained a few days before death included: blood urea nitrogen, 245 mg. per cent; proteinuria, 4 plus; hemoglobin, 8.5 gm. per 100 cc.; and red blood cell count, 2.94 million per cu. mm.

CASE 2. C. L., a twenty-five year old Negro man, was entirely well until February, 1945, when, while on active Army duty, he noticed swelling of his left leg. Proteinuria was discovered. He was sent to a number of Army hospitals. At times he suffered from facial edema and edema of both legs. In January,

1946, his urine showed 4 plus proteinuria, many granular casts and consistent microscopic hematuria. The blood urea nitrogen ranged between 27 and 54 mg. per cent. His blood pressure was 140/80 mm. Hg. He was discharged in July, 1946. Three months later he was hospitalized again because of edema of the face and legs; the blood pressure was 160/100 mm. Hg. His next and last admission was in October, 1949; the chief complaints were nausea, vomiting and headaches. Initially, his blood pressure was 250/110 mm. Hg; blood urea nitrogen, 56 mg. per cent; hemoglobin, 11.5 gm. per 100 cc. The urine showed 2 to 3 plus protein, numerous white blood cells and occasional red blood cells in the sediment.

During the next two months his red cell count dropped to 1.5 million per cu. mm. and the hemoglobin to 7.6 gm. per 100 cc. Transfusions of 1,500 cc. of whole blood raised his red cell count to 3.3 million and hemoglobin to 10 gm. per 100 cc. There was no evidence of blood loss during this period. Over the next several weeks his blood values dropped rapidly again and his blood urea nitrogen rose steadily. Red cell residue from 1,000 cc. whole blood was given when the peripheral blood values reached the previously mentioned low levels. He failed to improve, cardiac failure developed, and he died twenty-five days after his last transfusion. Autopsy revealed the changes of arteriolar nephrosclerosis and acute pyelonephritis.

Case 3. M. W., a thirty-five year old white man, was rejected for duty by the Army in 1943 because of high blood pressure. Weakness and severe headaches brought him to the hospital in July, 1945. His blood pressure was 170 to 200/140 to 146 mm. Hg. His urine contained 2 plus protein, 2 to 8 red blood cells per high power field, and a few coarsely granular casts. The plasma non-protein nitrogen was 28 mg. per cent, the red blood count was 5.1 million per cu. mm. and the hemoglobin was 15 gm. per 100 cc. The diagnosis of chronic glomerulonephritis was made.

Significant anemia began to develop during the last few months of 1948, along with an increase in plasma non-protein nitrogen to 100 mg. per cent. By January, 1949, his red blood count was 3.22 million per cu. mm., and hemoglobin 10.2 gm. per 100 cc.

Cardiac failure precipitated his third hospital admission in March, 1949. His red cell count had dropped to 1.65 million per cu. mm. and his hemoglobin to 5.2 gm. per 100 cc., with reticulocytes of 12.5 per cent and packed cell volume of 15 per cent. There was no history of bleeding during this period, and the stools were guaiac-negative. Digitoxin produced compensation after which he was given a transfusion of the red cell residue from 1,000 cc. of whole blood and discharged.

He returned one month later because of increasing weakness and diarrhea. His plasma non-protein nitrogen at this time was 210 mg. per cent, his red blood count was 1.84 million per cu. mm. and the hemoglobin was 5.1 gm. per 100 cc. packed red cell volume 15 per cent, and reticulocytes 9 per cent. His course was rapidly downhill; he died in congestive failure one week after admission.

Case 4. H. B., a thirty year old white man, was found at the age of nineteen to have an elevated blood pressure and an abnormal urine showing protein and red cell casts. A diagnosis of chronic glomerulonephritis was made.

His first hospitalization was in July of 1948, because of increasing weakness and headaches. Arteriolar narrowing was seen on funduscopic examination. Laboratory examinations revealed the following: red blood count, 4.58 million per cu. mm.; hemoglobin, 12.4 gm. per 100 cc.; plasma non-protein nitrogen, 56 mg. per cent; 3 plus proteinuria with 8 to 10 white blood cells and 2 to 3 red blood cells per high power field in the urine sediment. He was discharged on a low protein, low phosphorus diet and was much improved during the next six weeks.

In October, 1948, he was re-hospitalized in congestive failure. He still showed no striking anemia, his red cell count being 4.38 million per cu. mm. with 11.0 gm. per 100 cc. of hemoglobin. The urine was unchanged. His plasma non-protein nitrogen had risen to 100 mg. per cent. In spite of the administration of digitalis there was very little improvement in his cardiac status. He became rapidly anemic without any evidence of blood loss; over a period of several weeks his red count dropped to 2.0 million per cu. mm. and his hemoglobin to 6.7 gm. per 100 cc. A transfusion of 500 cc. of red cell residue produced some temporary improvement but he continued to have nausea, vomiting, dyspnea and orthopnea. He was discharged but returned in three weeks with increasing cardiac failure; he died three weeks after this admission. The autopsy diagnosis was chronic glomerulonephritis.

Case 5. B. B., a white man twenty-five years of age, had acute inflammation of several large joints at the age of twelve which was diagnosed as rheumatic fever. Several months later his urine became rust-colored. At the age of nineteen he was rejected by the Army because of proteinuria. Hypertension was noted shortly thereafter.

The first of seven hospital admissions was in February, 1948. Laboratory examinations at that time revealed the following: red blood count, 2.6 million per cu. mm.; hemoglobin, 9.5 gm. per 100 cc.; plasma non-protein nitrogen, 50 mg. per cent. The urine contained 1 to 3 plus protein, an occasional red cell, and hyaline casts. The blood pressure was 210/140 mm. Hg. Nausea, vomiting and severe headaches present at the time of admission disappeared after several weeks of bed rest, salt and protein restriction. He was given the red cell residue from 1,000 cc. of blood and his red count rose to 3.6 million per cu. mm.

The patient's third admission was in January, 1949,

at which time he had a marked anemia (1.9 million red blood cells per cu. mm.), nausea, vomiting and severe headaches. His plasma non-protein nitrogen was 109 mg. per cent. A transfusion of red cell residue from 1,000 cc. whole blood was given again.

He continued to run a gradual downhill course, and his final admission was in January, 1950. Cardiac failure was present for the first time and his blood non-protein nitrogen had risen to 120 mg. per cent. The patient had occasional convulsive seizures and visual hallucinations. He had repeated epistaxis. His course was rapidly downhill, and he died on March 6, 1950. The necropsy diagnosis was subacute and chronic glomerulonephritis.

CASE 6. J. S., a seventy year old white man, was first hospitalized in October, 1933, for a gastrointestinal examination. He gave a history of a peptic ulcer for forty years with intermittent difficulty. He was again hospitalized ten years later for epigastric pain. His red blood count was 3.47 million per cu. mm. with a hemoglobin value of 10.9 gm. per 100 cc. His plasma non-protein nitrogen was 89 mg. per cent. One month later his red blood count was 2.64 million per cu. mm.; his hemoglobin, 7.9 gm. per 100 cc.; his non-protein nitrogen, 73 mg. per cent; and his blood pressure, 170/80 mm. Hg.

From 1945 until the time of his death in 1950, his anemia and azotemia persisted without any striking changes. He received numerous transfusions with transient improvement in his strength. His plasma non-protein nitrogen remained elevated within a range of 58 to 117 mg. per cent.

Beginning in early 1950 he began to notice exertional dyspnea and edema of the ankles without any striking change in peripheral blood values. During the next few months he improved temporarily when digitalis was administered, but he died in April, 1950, in cardiac failure. There was no marked increase in his anemia during his terminal months. The autopsy diagnosis was chronic glomerulonephritis and arteriolar nephrosclerosis.

CASE 7. W. T., a fifty year old Negro man, had been found in 1936 to have proteinuria (1 plus to 4 plus) in addition to many white blood cells, red blood cells and casts in his urine. His blood pressure at that time was 120/80 mm. Hg. When he was hospitalized ten years later his blood pressure was 156/110 mm. Hg, and his urine showed proteinuria with 2 or 3 white blood cells per high power field in the centrifuged sediment. In 1948, twelve years after his initial visit, his blood pressure was 180/125 mm. Hg, his urine showed 2 to 4 plus protein, and his plasma non-protein nitrogen was 42 mg. per cent. A diagnosis of chronic glomerulonephritis was made.

The patient was hospitalized in November, 1949, because of precordial pain; no electrocardiographic evidence of myocardial infarction was obtained. From this time on his plasma non-protein nitrogen gradually rose from 50 to 93 mg. per cent. His blood values remained rather stable during the time he was followed up. One year before death an anemia of 3.0 to 3.7 million red cells per cu. mm. developed and continued without significant change. The patient was admitted to another hospital in January, 1950, with congestive failure and died three days later.

CASE 8. S. M., a Negro man sixty-one years of age, was first seen in December, 1948, at which time he complained of headaches for five years and edema of the ankles for two years. There was no history of previous ill health. His blood pressure was 175/115 mm. Hg, and his urine showed the following: specific gravity, 1.008; protein, 4 plus; 10 to 15 red blood cells per high power field, and an occasional granular cast. His plasma non-protein nitrogen was 42 mg. per cent. The diagnosis was hypertensive cardiovascular disease, nephrosclerosis and azotemia.

The patient was admitted to the Barnes Hospital in July, 1949, with essentially the same complaints and laboratory findings listed. He was given digitalis and there was moderate improvement in his condition. A normocytic normochromic anemia, first detected six months previously, was present. His red blood count remained between 2.0 and 3.0 million per cu. mm. The plasma non-protein nitrogen varied between 53 and 80 mg. per cent.

CASE 9. G. H., a fifty-three year old Negro man, was first seen in 1949 complaining of pain in the right flank, frequency, urgency and hematuria. Physical examination revealed an urethral stricture, residual urine and marked prostatic hypertrophy. Bacilluria, proteinuria and hematuria were present. In March, 1950 he was admitted to the urological service where the urine showed a fixed low specific gravity, 4 plus proteinuria, and numerous red and white blood cells. His blood pressure was 200/120 mm. Hg; examination of the fundi revealed "grade 3 changes," and his heart was enlarged.

Laboratory data were as follows: red blood count, 2.8 million cells per cu. mm.; hemoglobin, 7 gm. per 100 cc.; packed red cell volume, 23 per cent; white blood count, 5,000 per cu. mm.; and plasma non-protein nitrogen, 98 mg. per cent. The urine culture was positive for coliform organisms. After treatment with aureomycin® his temperature returned to normal, and the urine became sterile. However, the patient continued his downhill course and died in September, 1950. Autopsy diagnoses included chronic pyelonephritis with complete destruction of the parenchyma of the left kidney, chronic urethritis and arteriosclerosis of the renal arteries.

CASE 10. P. L., an eighteen year old boy, had had scarlet fever six years previously, without any known renal complications. At the age of sixteen he had facial puffiness, particularly around the eyes, and marked edema of the ankles and legs. His blood pressure at that time was 190/105 mm. Hg. The edema cleared in a few weeks. About one year later he noted the onset of severe headaches associated with blurring of vision in both eyes. Six weeks prior to hospital admission in April, 1950, transient ankle and periorbital edema again appeared. There was moderate exertional dyspnea. Examination of the fundi revealed arteriolar narrowing, arteriovenous nicking, old and fresh hemorrhages in both eyes, and scattered exudates. There was no evidence of congestive heart failure.

Laboratory data were as follows: red blood count, 2.3 million per cu. mm.; hemoglobin, 7.5 gm. per 100 cc.; and plasma non-protein nitrogen, 65 mg. per cent. The urine contained 2 plus protein, large numbers of red and white cells, no casts. There was fixation of specific gravity of the urine at 1.011. The diagnosis was chronic glomerulonephritis.

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Abnormal Hemoglobins in the Negroes of Surinam*

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STUDIES of the occurrence of abnormal hemoglobins have shown a remarkably high incidence of sickle cell hemoglobin [*1*] and of hemoglobin C [*2*] in certain areas of Africa. The suggestion has been made by Allison [*3*], Raper [*4*] and others that the presence of sickle cell hemoglobin confers some protection against Plasmodium falciparum malaria.

In recent years the introduction of filter paper electrophoresis has made possible the identification of a number of abnormal hemoglobins [*5*] and the distinction between sickle cell trait and sickle cell disease.§ This technic was employed to exploit an unusual opportunity to accumulate data on the incidence of abnormal hemoglobins in an area with endemic P. falciparum malaria, in Surinam, South America. The "Bush Negroes" of this area, the Djukas, are descendants of West African slaves brought to Surinam in the seventeenth and eighteenth centuries; these slaves escaped from their owners and established their own villages in the interior of Surinam. There has been little or no intermixture of the Djuka population with the South American Indians or with the "town Negroes" who are descendants of the slaves freed in the mid-nineteenth century. The history and customs of this population are discussed fully by Kahn [*6*].

The present study was designed to investigate the incidence of abnormal hemoglobins by filter paper electrophoresis; some data were also accumulated on the incidence of malaria in the Djuka population. As a part of the study two additional observations were made: (1) the extent of anemia in the population was appraised by determination of hemoglobin levels and erythrocyte counts, and (2) a concomitant study was made of the distribution of blood groups ABO and Rh.

§ The term sickle cell disease includes both sickle cell anemia and the genetic variants of sickling, e.g., sickle cell-hemoglobin C disease and thalassemia-sickle cell disease.

MATERIALS AND METHODS

The studies reported here were carried out on blood specimens obtained from 203 Djukas visiting the Out Patient Department of the hospital in Moengo, a bauxite mining community in the interior of Surinam. During field trips, additional blood specimens were obtained from 140 Djukas living in the villages of Langetabbatje and Petro Ondro in the interior of the country.

Of the 203 Djukas seen at the hospital, sixty-seven had no clinical complaints; twenty were pregnant; 116 complained of a variety of symptoms, the most common being diffuse aches and pains, chills and fever, and toothache. Tropical ulcers, yaws and traumatic injuries were also seen.

Malaria smears, blood grouping and hemoglobin determinations were carried out in the majority but not in all of these subjects. (See tables for numbers of subjects in each study.) Technical difficulties provided the only basis of rejection of subjects for these additional determinations.

Five milliliters of blood were drawn, and thick and thin smears for malaria were prepared immediately. The thick smears were examined for the presence of malarial parasites, and the species was distinguished

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on the thin smear. The density of each infection was estimated by the examination of fifty fields of a thin blood smear with an oil immersion lens. In fifty such fields, the presence of one parasite or less was considered a light infection; two to four parasites were considered a moderate infection; and five or more parasites were considered a heavy infection.* The remainder of the blood was added to oxalate and samples were removed for hemoglobin determination, red blood cell counts and blood typing. Hemoglobin levels were measured with a photoelectric colorimeter, the mean corpuscular hemoglobin was calculated, and in many cases erythrocyte morphology was inspected in blood smears. Erythrocytes from each specimen were washed and hemolyzed, and a solution of oxyhemoglobin was prepared for filter paper electrophoresis by a method described previously [7].† Erythrocytes were used for determination of ABO and Rh_0 (D) blood groups with refrigerated standard antiserums.

TABLE I

DISTRIBUTION OF HEMOGLOBINS IN THE DJUKA POPULATION

Number tested	343		
Number with Hemoglobin A only	284	(82.8%)	
Number with Hemoglobin A and Hemoglobin S	46	(13.4%)	—14.6%
Number with Hemoglobin S predominantly	4	(1.2%)	
Number with Hemoglobin A and Hemoglobin C	9	(2.6%)	

RESULTS

Types of Hemoglobin. The results obtained by filter paper electrophoresis of hemoglobin from 343 subjects are summarized in Table I. In fifty, or 14.6 per cent, hemoglobin S was found; forty-six of these fifty (13.4 per cent of the total studied) presented the pattern of sickle cell trait; in the remaining four the pattern of sickle cell disease was found. Nine (2.6 per cent) of the Djukas studied had hemoglobin C, all in the heterozygous form of hemoglobin C trait.

Malaria and Abnormal Hemoglobins. In the thick and thin blood smears prepared from venous blood specimens of 326 Djukas, sixty-four (20 per cent) showed malarial parasites. P. falciparum was identified in fifty-six of the positive smears; Plasmodium vivax was found in the remaining eight positive smears.

The incidence of malarial infection in various age groups (arranged by age in decades) may be seen in Table II. The highest incidence of infection occurred during the first ten years of life, reaffirming frequent observations that malarial infection rates are higher in children than in adults, and that in those who survive the infection, increasing age is accompanied by increasing immunity. Only forty-three children in the first decade of life were studied.

TABLE II

INCIDENCE OF MALARIA IN VARIOUS AGE GROUPS COMPARISON OF DJUKAS WITH HEMOGLOBIN A ONLY AND WITH HEMOGLOBIN A AND S

Age in Decades	Djukas with Hemoglobin A Only			Djukas with Hemoglobin A and S		
	Tested (no.)	Malaria Positive (no.)	Malaria Positive (%)	Tested (no.)	Malaria Positive (no.)	Malaria Positive (%)
I	36	15	42	7	4*	57
II	67	14	21	14	5	36
III	96	14	15	8	1	13
IV–VIII	71	8	11	14	0	..
Total	270	51	19	43	10	23

* One child had P. vivax parasitemia.

There was considerable variation in malarial infection rates in the three villages in which the studies were carried out. The incidence of malaria was lowest (12 per cent) among 220 Djukas living near Moengo; this low rate undoubtedly reflects the recent institution of malaria control measures in that area. In contrast, in fifty-two subjects in the villages of Langetabbatje and in fifty-four subjects in Petro Ondro, areas in which no measures for control of malaria have been undertaken, infection rates of 29 per cent and 41 per cent, respectively, were found.

An over-all malarial infection rate of 20 per cent was found in this Djuka population. Only a few persons with predominately hemoglobin S and with hemoglobin C trait were encountered; however, the incidence of malaria was about the same in all groups. (Table III.) In Table II the decreasing incidence of parasitemia with increasing age is shown to be approximately the same in "sicklers" and in "non-sicklers" in this small sample. Similarly, as shown in

* These criteria were used to classify the data in this study; it is recognized that severe P. falciparum infections may have parasite densities far in excess of any encountered in this study.

† Wet sealed sickling preparations were not obtained regularly and the possibility that a hemoglobin pattern interpreted as S hemoglobin might have been the rare D hemoglobin was not eliminated.

Table IV, no difference in density of malarial infection was noted between those without the sickle cell trait and in the small group of those with the sickle cell trait and malaria.

TABLE III
INCIDENCE OF MALARIA IN DJUKAS WITH VARIOUS TYPES OF HEMOGLOBIN

Type Hemoglobin	Tested (no.)	Malaria Positive (no.)	Malaria Positive (%)
Hemoglobin A only	270	51	19
Hemoglobin A and S	43	10	23
Hemoglobin S predominantly	4	1	25
Hemoglobin A and C	9	2	22
Total	326	64	20

TABLE IV
DENSITY* OF MALARIAL INFECTION

Data	Djukas with Hemoglobin A Only		Djukas with Hemoglobin S or A and S	
	No.	%	No.	%
Number tested for malaria	270	..	47	..
Light infection	32	12	7	15
Moderate infection	14	5	4	9
Heavy infection	5	2	0	..
No malaria found	219	81	36	77

* See test for definition of density of infection.

Blood Groups. The results of blood grouping are shown in Table V, and will be discussed subsequently.

Anemia. Anemia was defined as a hemoglobin level of less than 12.0 gm. per cent in all subjects, except children in the first decade of life and pregnant women [*8*]. Children under ten years of age were considered anemic if hemoglobin values were less than 10.0 gm. per cent, and pregnant women were classified as anemic only when the hemoglobin value was less than 9.0 gm. per cent. Hypochromia was defined as a mean corpuscular hemoglobin value of less than 25 $\mu\mu$g. except for pregnant women in whom only values less than 22 $\mu\mu$g. were considered abnormal.

As shown in Table VI, 74 per cent of the 278 persons in whom blood hemoglobin values and erythrocyte counts were determined were found to be anemic; and in 161 (58 per cent) the anemia was hypochromic. Of the twenty-seven children in the first decade of life who were studied 88 per cent had a hypochromic anemia; in women of reproductive age the incidence of hypochromic anemia remained high, while in adult men it diminished.

Of the 205 anemic subjects, five were found to have mild macrocytic anemias.

TABLE V
BLOOD GROUPS IN 279 DJUKAS

Blood Group	Djukas
O	55%
A	15%
B	26%
AB	4%
Rh_o(D)	98%

TABLE VI
INCIDENCE OF ANEMIA IN DJUKAS

Total number of Djukas tested..... 278
Subjects with anemia*..... 205 (73.7%)
Subjects with hypochromic anemia..... 161 (57.9%)

Age in Decades	Female			Male		
	Subjects (no.)	Anemia (%)	Hypochromic Anemia (%)	Subjects (no.)	Anemia (%)	Hypochromic Anemia (%)
I	10	100	90	17	100	87
II	40	78	55	26	85	69
III	66	68	52	33	57	48
IV	12	75	75	15	46	33
V	17	94	88	15	60	46
VI–VIII	14	86	43	13	62	38
Total	159	77	60	119	69	55

* Physiologic anemia of pregnancy was excluded.

COMMENTS

Types of Hemoglobin. The incidence of hemoglobin S in the Djukas (15 per cent) was significantly higher than in American Negroes (8 per cent) [*9*]; studies of West African Negroes have disclosed sickling rates as high as 19 per cent [*2*].

The incidence of hemoglobin C, 2.8 per cent, was comparable to the incidence found in studies

of Negroes in the United States [*10,11*]. An incidence ranging from 10 to 22 per cent has been found in Gold Coast Negroes [*2*]. We can offer no explanation of the difference in incidence of hemoglobin C, between Djukas and West African Negroes; the precise geographic home of the African forebears of the Djukas is, of course, not known.

Although definitive diagnostic testing for the presence of thalassemia in the Djuka population was not carried out, evidence from two cases of sickle cell disease suggested that the gene for thalassemia was also present. One subject, a two year old child, had the hemoglobin electrophoretic pattern of sickle cell disease (more than 75 per cent hemoglobin S) although no electrophoretic abnormality was demonstrable in the hemoglobin of her mother. A second subject with the electrophoretic pattern of sickle cell disease was a man in the fifth decade whose hemoglobin level was 13.2 gm. per cent with 6.2 million erythrocytes per cu. mm., his sole complaint was conjunctivitis. Both of these cases would better fit sickle cell-thalassemia disease than sickle cell anemia of the "homozygous" variety. Unfortunately, serum iron levels were not determined, and increase in the minor (A_2) hemoglobin component described by Kunkel [*12*] was not looked for; refined methods such as these would be needed to establish the frequency of the gene for thalassemia in a population whose hypochromic microcytic anemia and splenomegaly were probably largely related to parasitic infestation. However, thalassemia-sickle cell disease [*13*] has been reported in American Negroes, as has thalassemia itself [*14*], and the presence of the gene for thalassemia provided the best explanation for the findings in these two cases of sickle cell disease.

Malaria and Hemoglobin S. In recent years much conflicting evidence has accumulated concerning the possible protection afforded by the presence of sickle cell trait against P. falciparum infection. Probably the first such observation was made by Beet who found fewer instances of malaria among sicklers than among non-sicklers in Northern Rhodesia [*15*]. In 1949 Mackey suggested that in an area in which malaria is hyperendemic, sicklers have a better chance to survive to adult life than do non-sicklers [*16*]. However, Raper in a similar study was unable to demonstrate any difference in survival of sicklers and of non-sicklers [*17*]. In a more detailed study Allison [*3*] showed a lower infection rate in children with the sickle cell trait than in those without the trait.

Data supporting the theory that sickling protects against P. falciparum infection were obtained by Edington [*18*] who demonstrated a lower density of parasitemia in sicklers with malaria than in non-sicklers. Raper [*19*] in a study of 1,200 children again showed a lower density of parasitemia in infected sicklers. Deliyannis [*20*], who observed malaria in families of sicklers found a lower incidence of parasitemia in those with sickling than in the normal siblings. In a study of hospitalized children Raper [*21*] found more cerebral malaria in non-sicklers than in sicklers. On the basis of African mortality rates in childhood, Lehmann and Raper [*22*] have calculated that the selective advantage of sickling heterozygotes with respect to malaria could offset the loss of homozygous sicklers and thus account for the high rates of sickling in some African areas.

Other workers have failed to demonstrate that sicklers have any added protection against P. falciparum malaria. Moore et al. [*23*] in a study including both children and adults found essentially the same infection rates in sicklers and in non-sicklers. Foy and co-workers [*24*] found great variation in sickling rates (from 3 to 55 per cent) despite a low incidence of malaria in certain areas of India. Archibald and Bruce-Chwatt [*25*] were unable to show any relationship between the density of P. falciparum infection and sickling in African children.

Recent studies by Colbourne and Edington [*26*] and by Foy and co-workers [*27*] have shown that in some areas protection against P. falciparum infection appeared to be related to the presence of sickling while in other geographic areas no association could be found. Colbourne [*26*] suggests that protection against P. falciparum malaria could not be the sole mechanism responsible for the increased incidence of the sickling gene.

Allison [*3*] did direct P. falciparum inoculation experiments on fifteen sicklers and on fifteen non-sicklers in a malarial area of Africa. Of the non-sicklers positive blood smears developed in fourteen while of the sicklers, positive smears developed in only two. In contrast to these findings, Beutler et al. [*28*] performed direct P. falciparum inoculation studies in American Negroes who had never had had malaria and found no significant differences in parasite counts in sicklers and in non-sicklers at various intervals after

infection. To investigate the effect of acquired immunity, ten subjects were reinoculated four months later and again no significant differences were found between the two groups. The lack of demonstrable protection against P. falciparum in the non-immune sickling adult, shown in the experiments of Beutler [*28*], is an impressive finding and indicates that if any protection against P. falciparum is afforded by sickling, it must be of a specialized variety. However, the possibility that children with sickle cell trait are protected from lethal malaria still remains [*21,27*].

The present study was carried out on a Negro population which had lived in a malarial area of the New World for 300 years. A higher rate of sickling was found in this population than in American Negroes in the United States. There was no difference in the incidence of malaria in sicklers and in non-sicklers, but the population studied was small. The selective advantage of the person with sickle cell hemoglobin over the individual with hemoglobin A in a malarious area may exist only in early childhood; therefore the coexistence of sickle cell hemoglobin and P. falciparum malaria in adults does not provide an argument against the theory of selective advantage.

Blood Groups. The distribution of ABO and Rh blood groups in Djukas is shown in Table III; these results are comparable to those obtained in similar studies of West African Negroes [*29,30*]. American Negroes have a lower incidence of groups O and Rh_o, with a higher incidence of group A [*29,31*].

Anemia. The high incidence of hypochromic anemia in this population can probably be attributed in large part to the high incidence of intestinal parasites, particularly ancylostoma and ascaris [*32*]. Less iron deficiency was encountered in adult males than in children or in adult females, whose iron needs are greater; only five persons had erythrocyte macrocytosis and the anemia was mild in each case.

SUMMARY

1. A study of the incidence of abnormal hemoglobins among the Djuka population of Surinam revealed an incidence of sickle cell hemoglobin of 15 per cent and an incidence of hemoglobin C of 3 per cent. Two cases interpreted as sickle cell-thalassemia disease indicated the probable presence of the gene for thalassemia in the Djukas. The incidence of sickling and the distribution of the ABO and Rh_o (D) blood groups were comparable to those found in studies of the West African population; the incidence of hemoglobin C was significantly lower among the Djukas than among Gold Coast Africans.

2. An overall malarial infection rate of 20 per cent was found among the Djukas; 88 per cent of the malarial infections were caused by P. falciparum. The incidence of P. falciparum parasitemia was the same in sicklers as in non-sicklers. The number of children in the study was small [*43*]; sickling and P. falciparum malaria were found simultaneously in three of these children.

3. A high incidence of hypochromic anemia, presumed to be due to parasites, was encountered.

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Fever, Infection and Host Resistance in Acute Leukemia*

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FEVER and infection have been considered among the most common manifestations of the leukemias since the earliest reports by Craigie [*1*], Bennett [*2*], Virchow [*3*] and Ebstein [*4*]. In acute leukemia, fever has been attributed to the "general hypermetabolic condition of the patient" or to coexisting infection. Indeed, infection is so common in the natural history of the disease that the early literature is replete with reports incriminating a variety of bacteria, viruses and parasites as causative or precipitating agents [*6*]. Detailed definition of such clinical manifestations of acute leukemia as fever and infection has recently assumed great importance. This is related to the need for understanding the course of the disease during the evaluation of drugs with known or potential chemotherapeutic value, and in affording optimal patient care during the interval before these drugs exert their definitive effect.

As the first procedure in defining the problem of fever in acute leukemia it seemed necessary to delineate with modern bacterial, fungal and viral technics those febrile states which could be related to infection. A direct consequence of the information derived would be facts useful in the diagnostic and therapeutic management of the febrile leukemic patient. Accordingly a detailed review was made of the records of twenty patients with acute leukemia who had been treated at the Clinical Center, National Cancer Institute. A protocol was then designed to study fever and infection in acute leukemia. To assess certain factors of host resistance to infection, measurements were made of antibody response, serum proteins and the phagocytic activity of leukocytes. The studies pertaining to host resistance will be reported in detail in separate communications but a summary of the pertinent findings will be given in a subsequent portion of the paper.

METHODS AND MATERIALS

The study was begun on March 15, 1955 and terminated on March 1, 1956. The thirty-six patients with active acute leukemia who are included in this report were either patients in the Clinical Center at the commencement of the study or were admitted prior to the termination date of the study. Two additional patients were excluded because they died within twelve hours after their admission.

Patients were admitted to the Clinical Center as soon as the diagnosis of acute leukemia was made, and were included in the study as long as active hematologic disease persisted. Patients were not included in this study during intervening periods of partial or complete hematologic remission.

In each case the diagnosis of acute leukemia was made by examination of the bone marrow aspiration with supravital and fixed preparations employing Janus green and Giemsa stains, respectively. Sections of the bone marrow clot were stained with hematoxylin and eosin. The bone marrow criteria used to classify the activity of the disease were those accepted by the Clinical Studies Panel of the Cancer Chemotherapy National Service Center [*7*]. Hemoglobin, platelet count, leukocyte count and differential distribution were routinely obtained three times weekly. Drugs used in the treatment of acute leukemia during this period were 6-mercaptopurine and 4-amino-N^{10}-methyl-pterolyl-glutamic acid (methotrexate), corticotropin, cortisone and other structurally related steroids.

A fever was arbitrarily defined as a rectal temperature of 38.0°C. or higher. Rectal temperatures were

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taken of all patients a minimum of four times a day and were recorded in centigrade. Antipyretic drugs (other than steroids) and "prophylactic" antibiotics were not used. At least one of us examined each patient daily.

DIAGNOSTIC PROCEDURES

On admission certain laboratory procedures were performed routinely on afebrile patients. Bacterial and fungal cultures of the pharynx, and bacterial cultures of the urine and stool were obtained. If the patient had been receiving steroids a blood culture was performed. Specimens from the throat, stool, blood (serum and clot) were frozen for subsequent viral studies. Serum was also frozen for future use in comparative antibody studies and for protein electrophoresis. Roentgenograms of the chest and sinuses were obtained.

The diagnostic procedures which were employed when fever occurred depended on the presence or absence of symptoms or signs of a source of infection on physical examination.

Diagnostic Procedure for Fever Associated with Symptoms or Signs on Physical Examination of a Focus of Infection. In these instances specific cultures pertaining to the focus of infection were obtained (e.g. abscesses, sputum, pleural fluid, etc.) as were cultures of the pharynx, urine, stool and blood. The roentgenograms mentioned were routinely taken. Further follow-up procedures were repeated as necessary. Pertinent agglutination studies, cultures and roentgenograms were obtained after treatment and these were compared with the findings on admission and during active infection. After the results of the laboratory data became available it became apparent that this group could be divided depending upon whether or not a bacterial agent could be incriminated as a cause for the infection.

Diagnostic Procedure for Fever not Associated with Symptoms and Signs on Physical Examination of a Source of Infection. It was evident from our preliminary studies that it would be impractical to employ extensive laboratory procedures to investigate every temperature elevation when symptoms and signs of infection were absent. In these instances it was arbitrarily decided that the fever had to have certain qualifying features to warrant laboratory investigation. The qualifications were based on the degree of the highest daily temperature elevation and the duration of the fever. They were (1) a sudden spike in temperature to 40.0°c. or higher in a previously afebrile patient, or a temperature of 40.0°c. or higher on admission; (2) a minimum daily temperature of 38.5°c. for three consecutive days; or (3) a minimum daily temperature of 38.0°c. for five consecutive days. On the day these criteria were met cultures of the pharynx, blood, urine and stool were obtained, as were roentgenograms of the chest and sinuses, and specimens from the pharynx, blood and stool for virus isolation. Those patients in whom no focus of infection was found, even after laboratory studies, were considered to have a fever of undetermined origin. The diagnostic procedures outlined were repeated on the third and seventh day after the onset of the fever of undetermined origin and at a maximal interval of every seven days thereafter as long as the patient remained febrile.

Two other febrile reactions deserve comment. In a number of patients temperature elevations developed during or shortly after a blood transfusion or following administration of one of the antigens employed in the antibody studies. If the fever subsided within twenty-four hours and the patient remained afebrile, the temperature elevation was considered to have been causally related to one of these factors.

The specimens obtained from all patients for the elucidation of fever and infection were treated in the same fashion.

Bacteria. Gram-stain smears were routinely made from swabs of abscesses, sputum, pharynx, stool and urinary sediment (catheterized specimens from females and "clean" specimens from males). Other special staining technics were employed whenever indicated. To determine total flora and to make a semiquantitative estimate of the number of microorganisms present in each specimen the following detailed cultural methods were employed:

Pharyngeal and nasal specimens: Trypticase soy broth; rabbit blood agar plate.

Sputum: Thioglycolate broth; rabbit blood and desoxycholate agar plates.

Blood: Trypticase soy broth with 0.1 per cent agar, trypticase soy broth with 0.1 per cent agar and 0.1 per cent mucin, thioglycolate broth; trypticase soy agar pour plates (two).

Stool: Trypticase soy broth; tetrathionate brilliant green, and selenite F enrichment broths; salmonella-shigella (S-S), eosin-methylene-blue, trypticase soy and desoxycholate agar plates.

Urine sediment: Trypticase soy broth containing rabbit blood and a blood agar plate incubated

in 10 per cent carbon dioxide atmosphere; thioglycolate broth; desoxycholate agar plates.

Cerebrospinal, pleural, pericardial or abdominal fluid: Trypticase soy broth containing rabbit blood and a blood agar plate incubated in 10 per cent carbon dioxide atmosphere; thioglycolate broth.

Wounds, abscesses, etc: Thioglycolate broth; meat infusion broth; desoxycholate agar and blood agar plates incubated aerobically and anaerobically.

Tuberculous infections (suspected): Modified Jensen-Lowenstein medium and Youman's liquid medium.

Appropriate biochemical, serological and animal inoculation procedures were utilized in the complete identification of the organism isolated whenever possible.

Fungi. Specimens from the pharynx, nose, stool, urine or other suspected sites of infection were cultured on the following media: Thioglycolate and blood agar plate incubated at 37.0°C.; dextrose Sabouraud slant containing 1,000 units of penicillin and 1,000 μg. of streptomycin per ml., incubated at 30°C.; dextrose Sabouraud and rabbit blood agar slants containing no antibiotics, incubated at 30°C. If slants showed growth of fungus, other media and animal and biochemical procedures were employed for identification.

Viruses. Specimens including pharyngeal swabs, blood (clot), stool and autopsy material were frozen and were subsequently studied for evidence of virus infection. Except for preliminary freezing, all material was treated as described in a previous paper [*8*]. In brief, specimens were inoculated into animals, embryonated hens' eggs and tissue cultures. Adult and suckling mice were inoculated intraperitoneally and intracranially. Two serial passages in adult mice were made routinely. Seven and ten to twelve day old embryonated hens' eggs were inoculated intra-allantoically and observed for seven and two days, respectively. The fluid was then harvested and tested for hemagglutinating effect on chicken red cells. Two serial passages in chick embryos were also performed routinely and similarly tested. Monkey kidney and HeLa cell lines were used for tissue culture. One serial passage was made in tissue culture.

Roentgenograms. Routine views of the chest were the posteroanterior and left lateral. Because of the frequent finding of acute infection of the paranasal sinuses in our earlier experience, routine films included the Waters, Caldwell, open-mouthed Waters and lateral positions. These provided views of the ethmoid, sphenoid, maxillary and frontal sinuses.

ANTIMICROBIAL THERAPY

With fever in the presence of a focus of infection, antibiotic therapy was begun in a standardized fashion. Thus, for an initial clinical diagnosis of pharyngitis, acute tracheobronchitis, pneumonia and localized soft tissue infections, patients were given penicillin and streptomycin; for urinary tract infections, tetracycline; for gram-negative bacterial septicemias, tetracycline and streptomycin; for gram-positive coccal septicemias, penicillin. The daily dosages employed for tetracycline and for streptomycin were 40 mg./kg. to the nearest 0.5 gm. For children weighing more than 15 kg., and for adults, 2.4 million units of aqueous penicillin G were given daily in divided doses intramuscularly every six hours; for children weighing less than 15 kg., the daily dose of penicillin was 1.2 million units. In instances of gram-positive coccal septicemias, double the specified dose of penicillin was given every four hours.

When the results of the cultures became available, antimicrobial therapy was modified, depending upon the identity of the organism, its sensitivity, and the course of the infection. The modification included change in the dose of the antibiotics listed and, when indicated, the use of chloramphenicol, polymyxin, sulfadiazine, erythromycin, nitrofurantoin and bacitracin.

With fever in the absence of a demonstrable focus of infection, administration of penicillin and streptomycin was begun immediately after the aforementioned specimens and x-ray films were obtained. When the laboratory studies or roentgenograms subsequently indicated a specific infection, and when the combination of penicillin and streptomycin was ineffective, antibiotics were adjusted accordingly. If no focus of infection was demonstrated by the studies mentioned the patient was considered to have a fever of undetermined origin, and penicillin and streptomycin were continued for seven days. If the temperature remained above 38.0°C., penicillin and streptomycin were discontinued and tetracycline (dosage *vide supra*) was administered for an additional seven days. If the patient remained febrile after fourteen days of antibiotic therapy, all antibiotics were discontinued.

METHOD OF ANALYSIS

For the purpose of this study, a febrile episode was defined in terms of the relationship of fever to: (1) proved infection, (2) presumptive infection, or (3) fever of undetermined origin.

TABLE I
CLASSIFICATION OF PATIENTS WITH ACUTE LEUKEMIA BY TYPE, AGE* AND SEX

Type of Leukemia	No. of Patients	Sex	
		Male	Female
Lymphocytic	29	15	14
Children	18	11	7
Adults	11	4	7
Granulocytic	7	3	4
Children	1	0	1
Adults	6	3	3
Total	36	18	18

* Patients less than fifteen years of age at the time of admission to the study are classified as children. Patients past their fifteenth birthday are classified as adults.

Proved infection: In these episodes (1) fever occurred in the presence of a focus of infection; (2) a causative agent was isolated; and (3) in those patients who survived the infection the signs and symptoms of the infection subsided concurrently with the disappearance of the causative organism.

Presumptive infection: In these episodes (1) fever occurred in the presence of a focus of infection, but (2) a specific causative agent could not be identified.

Fever of undetermined origin: In these episodes (1) fever occurred in the absence of any identifiable focus of infection, and (2) no infectious agent was isolated.

RESULTS

The thirty-six patients included in this study are classified in Table I by type of leukemia, age and sex. The period in which each patient was observed in the Clinical Center with active leukemia ranged from 2 to 196 days, the average being 72.6 days and the median 88 days. Thirty-three of the patients (91.7 per cent) were observed for thirty days or more, and twenty-one (58.3 per cent) for sixty days or more. In general, patients with granulocytic leukemia were under observation for longer periods than those with lymphocytic leukemia, the median days of observation being ninety-two and sixty-four days, respectively.

The data in Table II indicate that proved and presumptive infections were related to fifty-nine of the ninety-two (64.1 per cent) febrile episodes. However, because of their longer duration, fever of undetermined origin accounted for 55.5 per cent of the 971 febrile patient days.

The distribution of the type of febrile episodes in the thirty-six patients is given in Table III. Two patients* had no febrile episodes. Six patients had seven febrile episodes associated with fever of undetermined origin, fifteen patients had thirty-five febrile episodes attributed to infection (proved and presumptive), and in the remaining thirteen patients both infection and fever of undetermined origin accounted for fifty febrile episodes.

In Table IV the influence of age and type of acute leukemia on the rate and distribution of febrile episodes is analyzed. Since the number of patients in each of the four groups differed, and since the duration of observation per patient varied, the number of febrile episodes per 100 patients days is used as the standard of comparison for rate. Age and type of leukemia did not significantly affect the rate of febrile episodes in the four groups of patients. Age, and not morphologic type of leukemia, was correlated with the distribution of febrile episodes. In adults, the proportion of febrile episodes related to infection (proved and presumptive) was significantly greater than in children ($P = .02$ chi-square test). Conversely, the proportion of febrile episodes related to fever of undetermined origin in adults was significantly less than in children.

The over-all experience with fever in the thirty-six patients is summarized in Table V. It is of interest that fever occurred in almost 50 per cent of the total 2,614 patient days. Fever could not be classified in 11.6 per cent of the febrile patient days. For the most part the unclassified category represents isolated temperature elevations which were not accompanied by signs or symptoms of infection and were not of sufficient degree or duration to qualify for laboratory investigation according to the protocol.

To exclude the possibility that a few patients

* Both patients were adults. One had granulocytic, the other lymphocytic leukemia. They were observed for forty-four and forty-five days, respectively.

TABLE II

CLASSIFICATION AND AVERAGE DURATION OF FEBRILE EPISODES

Febrile Episode Resulted From	No. of Febrile Episodes	Per cent of Febrile Episodes	Febrile Patient-Days		Average No. Days per Episode
			No.	Per cent	
Proved infection	44	47.8	308	31.7	7.0
Presumptive infection	15	16.3	124	12.8	8.3
Fever of undetermined origin	33	35.9	539	55.5	16.3
Total	92	100.0	971	100.0	....

contributed the majority of the febrile days of those reported for the entire series of patients, the data were recalculated on the basis of percentage febrile days per patient; these percentages were then averaged. With this method the average of the per cent febrile days per patient is 44.9 per cent, which is in excellent agreement with the value of 44.5 per cent, the per cent of febrile days in the entire study. Similar calculations for each category of fever yielded results in substantial agreement with those reported in Table v.

FEBRILE EPISODES ASSOCIATED WITH PROVED INFECTION

The major clinical illnesses associated with the forty-four febrile episodes attributed to proved bacterial infections are listed in Table VI. Ten of the fifteen episodes of pharyngitis occurred in eight children, and the remaining five episodes occurred in three adults. The eleven episodes of pyelonephritis and cystitis occurred in six adults. One of these patients was a man. Shortly after the institution of antimetabolites he developed fever, and severe left lumbar pain which radiated to the groin. Microscopic examination of an uncentrifuged urine specimen showed many uric acid crystals. An intravenous pyelogram demonstrated evidence of hydronephrosis and pyelonephritis in the left kidney. Klebsiella cloacae was obtained from the urine in pure culture. After appropriate therapy the symptoms and signs abated, the patient became afebrile, and urine cultures became negative. Subsequent intravenous pyelograms showed restoration of normal renal pelvic contour. Four women had signs and symptoms of pyelonephritis on admission and in three of them recurrences developed during the course of hospitalization. In the fifth woman, extensive retroperitoneal hemorrhage produced obstruction of the left ureter and subsequent pyelonephritis.

Eight of the fourteen episodes of bacteremia were not associated with other apparent clinical infections. In two of these, however, a focus was found later. In one patient, acute appendicitis discovered at autopsy was the probable source of a Cl. perfringens septicemia. In the second patient M. pyogenes var. aureus was cultured from the blood and from an indwelling femoral catheter. Two of the three episodes of cellulitis were caused by Ps. aeruginosa and one by M. pyogenes var. aureus, coagulase-positive. Endometritis was observed in one patient. A vaginal pack was inserted because of profuse hemorrhage prior to admission to the Clinical Center. Before this pack was removed a ful-

TABLE III

DISTRIBUTION OF NINETY-TWO FEBRILE EPISODES IN THIRTY-SIX PATIENTS*

No. of febrile episodes associated with proved or presumptive infection	0	1	2	3	4
5	1				
4	2				
3	3	1	3		1
2	4	1	1	1	
1	5	2	2	1	
0	2	5	1		

No. of febrile episodes associated with fever of undetermined origin

* Numbers within square represent number of patients.

TABLE IV

DISTRIBUTION OF FEBRILE EPISODES ACCORDING TO TYPE OF LEUKEMIA AND AGE

Type of Leukemia	No. of Patients	No. of Febrile Episodes	Febrile Episodes per 100 Patient-Days	Per cent of Febrile Episodes Due to			
				Proved Infections	Presumptive Infections	Total Infections	Undetermined Origin
Lymphocytic	29	66	3.6	43.9	18.2	62.1	37.9
Children	18	44	3.6	31.8	20.5	52.3	47.7
Adults	11	22	3.8	68.2	13.6	81.8	18.2
Granulocytic	7	26	3.2	57.7	11.5	69.2	30.8
Children	1	7	3.6	14.3	28.6	42.9	57.1
Adults	6	19	3.1	73.7	5.3	79.0	21.0
Total	36	92	3.5	....	....	....	....

TABLE V

FREQUENCY AND CLASSIFICATION OF FEVER IN THIRTY-SIX PATIENTS WITH ACUTE LEUKEMIA

Data	No. of Days	No. of Febrile Patient Days	Per cent of All Days under Observation	Per cent of Days with Fever
Frequency of Fever				
Patient days without fever	1,452	...	55.5	.....
Patient days with fever	1,162	...	44.5	100.0
Total	2,614	...	100.0	.....
Classification of Fever				
Proved infection	....	308	.....	26.5
Presumptive infection	....	124	.....	10.7
Fever of undetermined origin	....	539	.....	46.4
Blood transfusion	....	33	.....	2.8
Immunization	....	26	.....	2.2
Unclassified	....	132	.....	11.4

minating endometritis developed with septicemia caused by E. coli and the patient died.

The episode of enteritis was due to a mixed infection with M. pyogenes var. aureus and C. albicans, and followed the use of tetracycline. The episodes of pneumonia and otitis media were not unusual.

The bacteria responsible for the forty-four infections are listed in Table VII. Of interest is the predominence of M. pyogenes var. aureus, coagulase-positive, and gram-negative rod-form bacteria. Relatively non-pathogenic organisms were capable of causing infection in patients with acute leukemia. For example, two episodes of septicemia were due to M. pyogenes, var. albus, coagulase-negative. (Contamination was unlikely, since in both instances the organisms were present in repeated cultures.)

In summary, twenty of the forty-four (45.5 per cent) bacterial infections were caused by gram-positive organisms. Four of the twenty cases ended in death. Twenty-four of the forty-four (54.4 per cent) bacterial infections were caused by gram-negative organisms. Six of these twenty-four cases ended in death. Of the three most frequently encountered organisms, Ps. aeruginosa was the most difficult to treat, death occurring in three of five (60.0 per cent) cases.

E. coli was responsible for fourteen infections; three cases ended in death (21.4 per cent). M. pyogenes var. aureus, coagulase-positive, caused thirteen infections, and death resulted in two (15.4 per cent) cases.

TABLE VI
MAJOR CLINICAL ILLNESS ASSOCIATED WITH FORTY-FOUR PROVED BACTERIAL INFECTIONS

Illness	Without Bacteremia	With Bacteremia	Total
Pharyngitis	14	1	15
Pyelonephritis and/or cystitis	10	1	11
Septicemia	..	8	8
Pneumonia	2	2	4
Cellulitis	2	1	3
Endometritis	0	1	1
Enteritis	1	0	1
Otitis media	1	0	1
Total	30	14	44

TABLE VII
BACTERIA ISOLATED IN FORTY-FOUR FEBRILE EPISODES DUE TO PROVED INFECTION

Name of Bacterium	No. of Febrile Episodes
E. coli	14
M. pyogenes var. aureus, coagulase-positive	13
Ps. aeruginosa	5
M. pyogenes var. albus, coagulase-negative	3
D. pneumoniae	2
Klebsiella sp.	2
Proteus sp.	2
β Hemolytic streptococcus	1
Cl. perfringens	1
Paracolon	1

FEBRILE EPISODES ASSOCIATED WITH PRESUMPTIVE INFECTION

The major clinical diagnoses made in the fifteen presumptive infections are listed in Table VIII. The majority of these cases represented pneumonias confirmed by chest roentgenograms. None of the comparative cold agglutinin titers which were obtained showed significant rises. Both episodes of acute tracheobronchitis occurred in children. In two patients the area of cellulitis was extensive, involving the entire forearm in one, and the buttock and upper thigh in the other. The diagnosis of acute sinusitis was confirmed by the appearance of air-fluid levels on roentgenograms of the sinuses. The diagnosis of lymphadenitis was made on the basis of enlarged, exquisitely tender epitrochlear and axillary lymph nodes accompanied by prominent soft tissue reaction and fever. In all the presumptive infections, control of the infection and defervescence appeared to be in temporal relation to antibiotic therapy.

TABLE VIII
CLINICAL ILLNESS ASSOCIATED WITH FIFTEEN PRESUMPTIVE INFECTIONS

Illness	No. of Febrile Episodes
Pneumonia	8
Tracheobronchitis	2
Cellulitis	2
Sinusitis	2
Adenitis	1
Total	15

FATALITY RATE IN PROVED AND PRESUMPTIVE INFECTIONS

Table IX depicts the fatality rate in proved and presumptive infections. While the figures in Table IX indicate that 22.7 per cent of the febrile episodes ended fatally, the importance of infection in patients with acute leukemia is further emphasized by the following: At the conclusion of the observation period on March 15, 1956, fourteen of the thirty-six patients in the study were dead. Of these fourteen, ten (71.4 per cent) died with overwhelming infection.

FEBRILE EPISODES ASSOCIATED WITH FEVER OF UNDETERMINED ORIGIN

In the remaining thirty-three febrile episodes no infectious agent could be incriminated for the febrile episode despite systematic bacterial, fungal and viral studies. In addition to the studies performed according to protocol, a variety of other tests were obtained whenever there was any historical or clinical suggestion of an infectious disease that might have been responsible for the fever. These included, for example, serologic determinations for leptospirosis, brucellosis,

tularemia, Q fever, salmonellosis, and bone marrow cultures for bacteria, fungi and viruses.

As previously described, all patients with fever of undetermined origin were treated initially with penicillin and streptomycin and if there was no response, with tetracycline. "Response to antibiotics" was arbitrarily defined as defervescence within ninety-six hours after their institution, with persistence of the afebrile state thereafter. An example of a response to antibiotics is shown in Figure 1.

TABLE IX
FATALITY RATE IN FEBRILE EPISODES DUE TO PROVED AND PRESUMPTIVE INFECTION

Data	No. of Febrile Episodes	No. of Febrile Episodes Ending Fatally	Per cent Fatality
Proved Infection			
Without bacteremia	30	3	10.0
With bacteremia	14	7	50.0
Total	44	10	22.7
Presumptive Infection			
Total	15	0	0.0
Proved and Presumptive Infection			
Without bacteremia	45	3	6.7
With bacteremia	14	7	50.0
Total	59	10	16.9

An analysis is presented in Table x of the response of fevers of undetermined origin to antibiotics and the relation of that response to steroid therapy. Of the thirty-three fevers of undetermined origin, twenty-eight occurred in patients who were not receiving steroids or who were on small maintenance doses of steroids. Fourteen of these twenty-eight fevers of undetermined origin responded to antibiotics. Those patients who became afebrile on tetracycline were rechallenged with penicillin-streptomycin to exclude the possibility of drug fever. In none of these instances did fever recur. Five children with acute lymphocytic leukemia accounted for the remaining five fevers of undetermined origin. Each received large doses of steroids equivalent to 5 mg./kg. body weight of hydrocortisone. (Because of a history of penicillin hypersensitivity, one patient was initially treated with tetracycline.) All five became afebrile within ninety-six hours.

Of the fourteen fevers of undetermined origin which persisted after antibiotic therapy, nine occurred in children with lymphocytic leukemia, three in adults with lymphocytic leukemia, one in a child with granulocytic leukemia, and one in an adult with granulocytic leukemia. There was considerable variation in the temperature patterns in these instances. Three examples of such variation are given in Figures 2, 3 and 4. The duration and termination of the fevers of undetermined origin are summarized in Table xi.

TABLE X
EFFECT OF ANTIBIOTICS IN THE TREATMENT OF FEBRILE EPISODES RELATED TO FEVER OF UNDETERMINED ORIGIN: RELATION TO STEROID THERAPY

	No Steroids	Maintenance Steroids	Therapeutic Steroids
Response to antibiotics	10	4	5
Penicillin, streptomycin	6	2	4
Tetracycline	4	2	1*
No response	12	2	0
Total	22	6	5

* See text.

TABLE XI
DURATION AND TERMINATION OF FOURTEEN FEBRILE EPISODES RELATED TO FEVER OF UNDETERMINED ORIGIN THAT DID NOT RESPOND TO ANTIBIOTICS

No. of Febrile Episodes	Median Duration after Antibiotic Therapy (days)	Termination
8	20	Development of bacterial infection
3	23	Defervescence related to subsequent use of steroids or antimetabolites
2	47	Febrile, conclusion of study
1	2	Death, intracerebral hemorrhage
14	21	

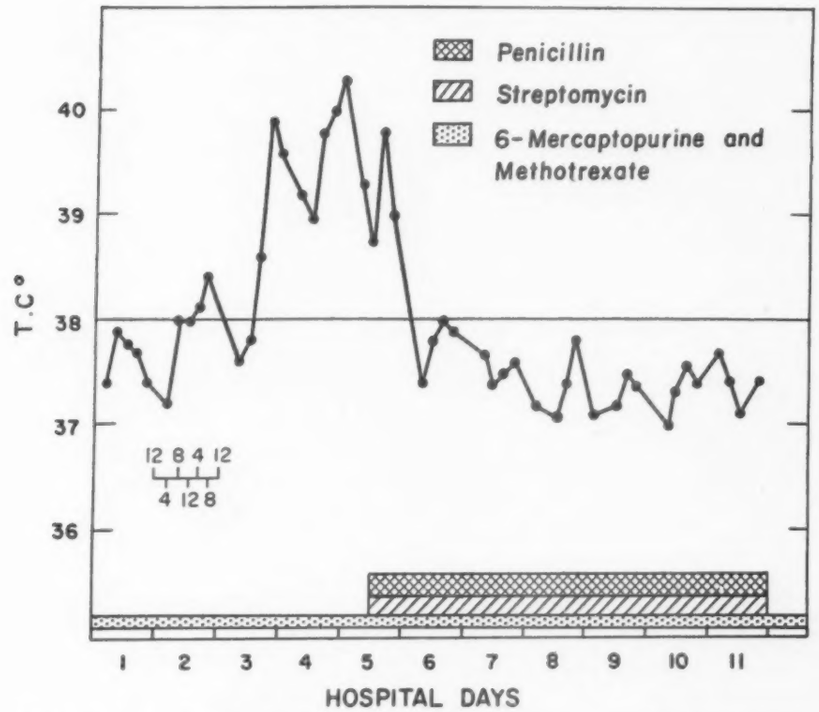

FIG. 1. A. L. L., a five year old white girl. Defervescence—fever of undetermined origin (F.U.O.)—in temporal relationship to antibiotic therapy.

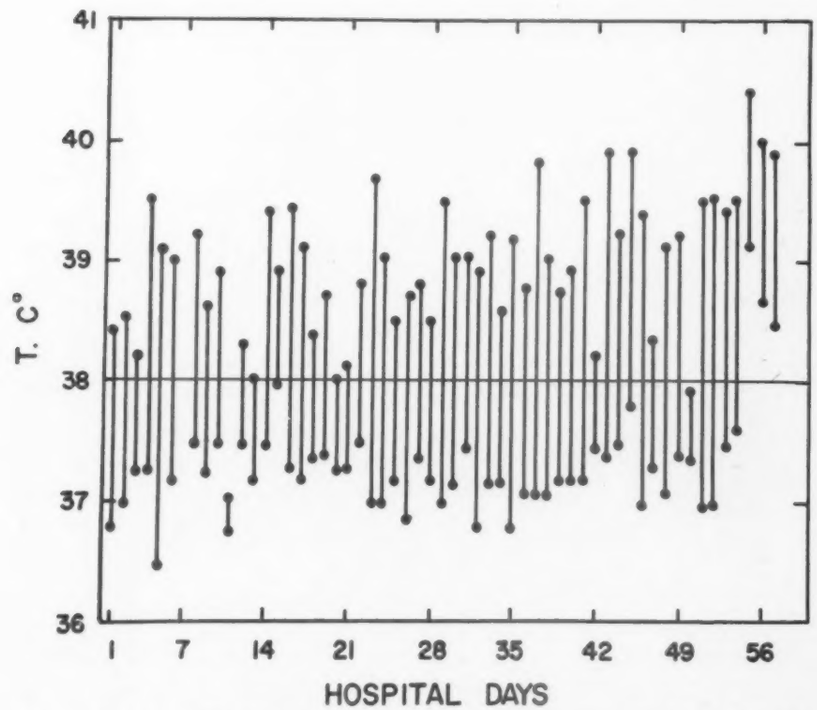

FIG. 2. A. L. L., a thirty-four year old white woman. Intermittent fever (F.U.O.) in acute leukemia.

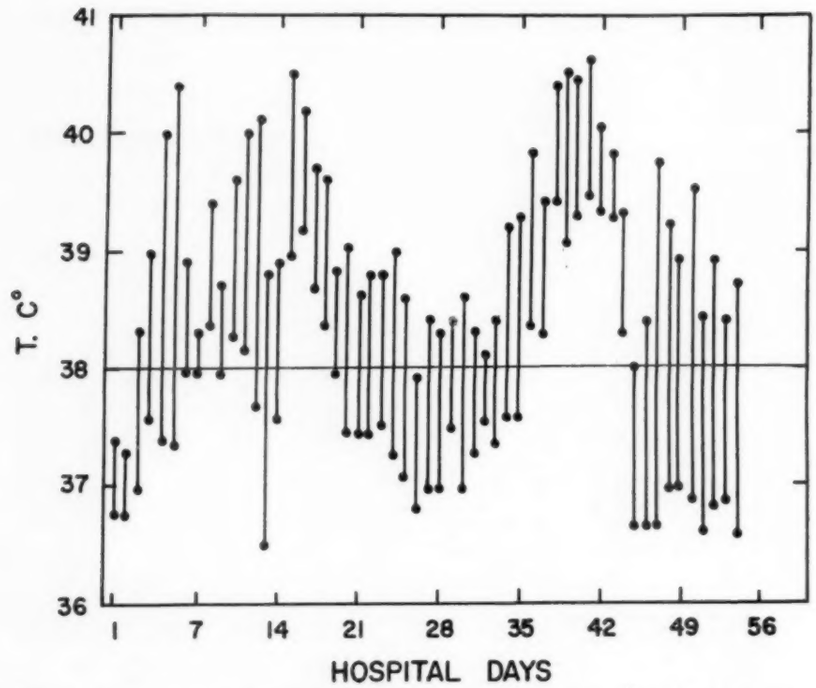

FIG. 3. A. L. L., a seven year old white girl. Cyclical fever (F.U.O.) in acute leukemia.

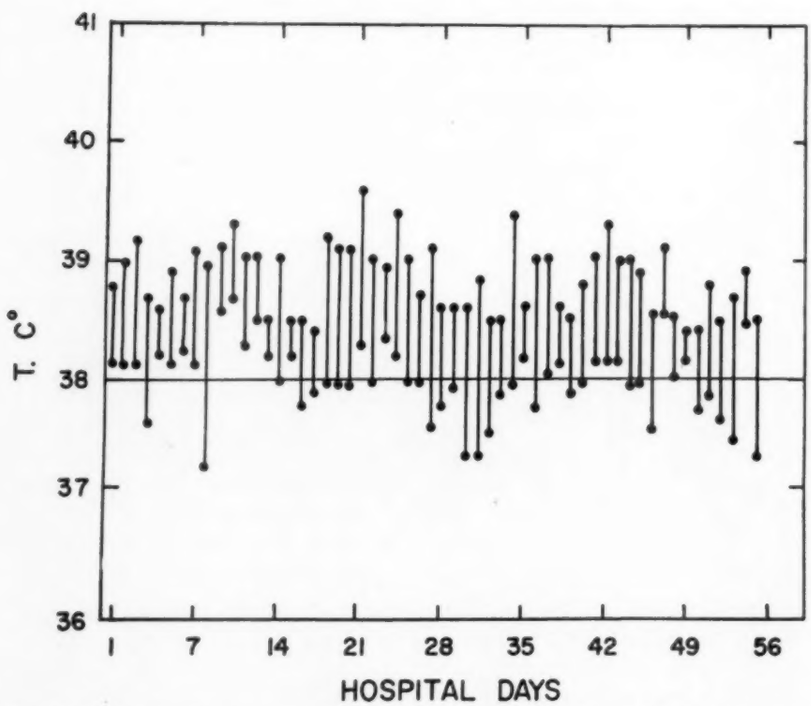

FIG. 4. A. G. L., a sixteen year old Negro girl. Continuous fever (F.U.O.) in acute leukemia.

Frequently, these fevers terminated in serious bacterial infections. There was no way to establish whether this represented the eventual isolation of the bacteria which were responsible for the febrile episode from its onset, or the superimposition of bacterial infection during a fever

TABLE XII
INITIAL IMPRESSIONS AND FINAL DIAGNOSES IN NINETY-TWO FEBRILE EPISODES

Initial Findings on Physical Examination	No. of Febrile Episodes	Final Diagnosis		
		Proved Infection	Presumptive Infection	Fever of Undetermined Origin
Focus of infection	48	36	12	..
No focus of infection	44	8	3	33
Total	92	44	15	33

of undetermined origin. In two febrile episodes the subsequent use of steroids was associated with defervescence although a hematologic remission was not induced. In one patient with fever of undetermined origin defervescence coincided with hematologic remission following combined 6-mercaptopurine and methotrexate therapy.

None of the fevers of undetermined origin in this group of patients appeared to be due to sensitivity to the antimetabolites used, as reported by Hyman et al. [9]. However, while on maintenance therapy during complete hematologic remission an unexplained fever did develop in one patient. This fever subsided soon after 6-mercaptopurine and methotrexate were discontinued.

The possibility of a neurogenic origin for persistent fever of undetermined origin was entertained. Sections of the hypothalamus were made in six of the ten patients who died during the study. Three of the six patients had fevers of undetermined origin, and in the other three, fever attributed to bacterial infection occurred during their illness. In sections from these six patients there were minimal non-specific acute neuronal changes characterized by nuclear pyknosis and karyorrhexis. In five of the six patients there was no evidence of leukemic infiltrate or hemorrhage in the hypothalamus. One patient was found to have perivascular leukemic cuffing in the meninges in the region of the hypothalamus without direct involvement of the brain substance. This patient, however, had had only one febrile episode related to proved infection early during hospitalization. In this sample, therefore, fevers of undetermined origin could not be attributed to lesions in the hypothalamus.

From the standpoint of patient care, it is to be emphasized that the absence of a focus of infection on physical examination does not rule out infection as a cause of fever in acute leukemia. (Table XII.) Of the eleven proved and presumptive infections discovered by laboratory procedures, positive blood cultures were obtained in eight; in three, roentgenographic evidence of pneumonia was apparent. Although the development of an infection in a patient with a fever of undetermined origin was not infrequently heralded by a qualitative or quantitative change in the temperature pattern, there was no reliable method for excluding the possibility of infection other than laboratory studies, particularly blood cultures and chest films.

TABLE XIII
FUNGUS INFECTIONS IN ACUTE LEUKEMIA

Total number of patients studied for fungi	36
No. of patients, fungi not isolated	18
No. of patients, fungi isolated	18
Patients with no signs of fungus infection	7
Patients with signs of fungus infection	11
Fungus infection temporally related to antibiotics	6
Fungus infection unrelated to antibiotics	5

TABLE XIV
FUNGUS INFECTIONS IN ACUTE LEUKEMIA

Name of Fungus	Type of Infection	
	No Signs of Infection	With Signs of Infection
C. albicans	1	7
C. albicans and stellatoidea	1	2
C. albicans and tropicalis	0	1
C. stellatoidea	2	1
C. tropicalis	2	0
Geotrichum	1	0
Total	7	11

FUNGUS INFECTIONS

Our experience with fungus infections in the thirty-six patients is summarized in Table XIII and the fungi isolated are listed in Table XIV.

In eight of eleven patients with characteristic clinical evidence of fungus infection, Candida was confined to the pharynx. Two patients had moniliasis of the pharynx, skin and bladder, and in these two patients eventual control of the fungus infections was related to hematologic remission rather than to the antifungal therapy administered. The remaining patient had monilial enteritis. Candida was not recovered from the blood of any of these patients. In five of the eleven patients clinical evidence of fungus disease appeared without any temporal relationship to antimicrobial therapy.

"HOST RESISTANCE" IN ACUTE LEUKEMIA

The frequent occurrence of bacterial infection during the course of acute leukemia led to a study of cellular and humoral defense mechanisms. Measurements were made of antibody response and serum proteins [*10*], and phagocytic activity of leukocytes [*11*]. Although as a group the antibody response of leukemic patients was less than that of a normal control group, some individual leukemic patients demonstrated excellent response to challenge antigens. The antibody response in a given patient could not be correlated with the frequency of bacterial infections. Although non-specific changes were observed in the serum proteins, significant abnormalities, particularly in the globulin fraction, did not occur. No difference in phagocytic activity was observed in the mature neutrophils during active leukemia in the absence or presence of infection, or during remission, when compared to normal controls. Phagocytosis by immature cells of both the lymphocytic and granulocytic series was negligible. Bacterial infection was preceded by, but did not necessarily follow, a dynamic fall of the order of 50 per cent or more in the mature neutrophil counts and not by a change in numbers of other leukocytes. The absolute mature neutrophil count when infection eventually developed varied from patient to patient. Indeed, not infrequently infection occurred at significantly different mature neutrophil levels within the same patient.

COMMENTS

The results of close and relatively long term observation of thirty-six consecutive patients studied in a uniform fashion yielded information which may be applicable to other groups of patients with acute leukemia. Nevertheless, certain uncontrollable factors have necessarily modified the relative frequency of fever and infection reported. The total period of active disease could not be observed in all patients. Antileukemic and antimicrobial therapy profoundly influenced the course of the entire illness, and in particular, fever and infection. Finally, the experimental design was such that approximately 10 per cent of the febrile patient days did not meet the criteria of the protocol and were therefore unclassified.

The age of the patient and not the morphologic type of acute leukemia was correlated with the relative frequency of febrile episodes due to infection and fever of undetermined origin. In adults, fever related to infection was relatively more common than fever of undetermined origin, while the reverse was true among children. It is well established that age is similarly associated with other features of acute leukemia such as morphologic type, bone involvement and response to antileukemic therapy. In both age groups, however, the febrile episodes due to infection were sufficiently common to suggest that significant episodes of fever in the patient with acute leukemia should be considered due to infection until proved otherwise. In the presence of a focus of infection, customary diagnostic procedures may be employed. In the absence of signs and symptoms, blood cultures and chest roentgenograms are the most valuable laboratory aids in detecting infection.

The bacterial infections encountered in our patients were somewhat unusual in a number of respects. Relatively avirulent organisms caused infectious disease. Irrespective of the type of bacterial organism, the clinical infections produced were generally of a serious nature. Although only the major clinical illness associated with bacterial infections are listed in Table VI, multiple sites of infection often occurred. (Thus, for example, in one patient who is listed with a "primary" infection of cellulitis and septicemia, pneumonia and pharyngitis subsequently developed.) Ultimate survival from infection depended not only on the nature of the organism and its antibiotic sensitivity but also on the degree of activity of a leukemic process. In two patients eventual control of infection was related to hematologic remission rather than to the antimicrobials administered; conversely, death with fulminating infection not uncommonly occurred even though the bacterial organisms were sensitive *in vitro* to a variety of anti-

microbial agents. Because of the markedly deranged hematopoietic system, the typical leukocytic response to bacterial infection usually did not occur either in specific sites of infection, or in the blood. In soft tissue lesions, pharyngeal exudate and urinary sediment, the absence of the mature neutrophil was characteristic. Bacterial phagocytosis by leukemic cells was not seen. These observations were consistent with those of Jaffe [*12*]. After examination of autopsy material he concluded that when granulopoesis in the leukemic patient was "exhausted," defense reaction did not occur, and that immature cells did not play a significant role in local defense. It was surprising that, in spite of such profound alterations in cellular defense mechanisms, the febrile episode fatality rate in proved infections was only 22.7 per cent. However, in this group of patients, as in others [*13,14*], overwhelming infection ranked with hemorrhage as a leading cause of death.

The administration of "prophylactic" antibiotics is becoming increasingly popular in a variety of diseases in which susceptibility to infection is increased. Many of the infections encountered in this study (e.g. those due to penicillin-resistant staphylococci, pseudomonas, etc.) probably would not have been prevented by the usual dose and type of antibiotic used in prophylaxis. Moreover, the many complications that may result from alterations of normal flora and the development of superinfections are to be particularly feared in acute leukemia. It is our impression, therefore, that long term prophylactic antibiotic therapy may not be advisable in patients with acute leukemia.

Because of their nature, it is believed that all the presumptive infections were probably of bacterial origin. The possibility exists, of course, that the cause of these infections (particularly the pneumonias) was other than bacterial, even though resolution of the lesion and defervescence temporally coincided with antibiotic therapy. Clinically, the presumptive infections, as a group, were not as severe as the proved infections.

The temporal response of fourteen fevers of undetermined origin to antibiotic therapy suggests these may have been due to undiscovered bacterial infection. Nevertheless, we hesitate to draw a cause-and-effect conclusion in these instances since other factors, such as antileukemic therapy or variations in the disease, may have played a more than coincidental role. Excluding the five febrile episodes in which the patients received large ("therapeutic") doses of steroids, defervescence occurred in temporal relation to antibiotic therapy in half of the remaining fevers of undetermined origin. This suggests that a course of antimicrobial therapy for seven to fourteen days may be of value. Because of the frequent development of clinical and laboratory evidence of infection after a fever of undetermined origin had persisted for days, repeated diagnostic procedures, particularly blood cultures and chest films, are indicated at weekly intervals.

The cause of the persistent fever in the patients who had no demonstrable source of infection remains obscure. Although viral illnesses certainly occur in leukemia [*15,16*] there was no clinical evidence of viral disease in these patients and attempts to isolate viruses were unsuccessful. During this period, when the specimens from the thirty-three patients with febrile episodes related to fevers of undetermined origin were being studied, viruses were isolated in this laboratory from fresh specimens from non-leukemic patients with clinical evidence of viral disease. Isolation attempts by tissue culture were made a second time on the specimens from patients with a fever of undetermined origin with similar negative results. Although freezing of specimens before inoculation is customary in most virus laboratories, the possibility exists that the process of freezing and thawing may have been partially responsible for the failure to isolate viruses from the specimens from leukemic patients as compared with those from non-leukemic patients [*8*].

It has been suggested that a factor common to all febrile disease is tissue injury [*17*]. Certainly in the patient with acute leukemia rapid turnover and destruction of tissue occurs. If fever of undetermined origin in acute leukemia is related to tissue injury, then the reason for such fever to develop only in certain patients is not readily explicable from our data. Fever of undetermined origin could not be correlated with leukocyte count, severity of anemia, hemorrhage or subsequent response to antileukemic therapy.

As with fever of undetermined origin, the basic mechanism whereby resistance to infection is impaired in acute leukemia remains unknown. It is to be emphasized that the susceptibility to bacterial infection cannot be entirely ascribed to dynamic changes in the number of circulating mature neutrophils in the individual patient. It is conceivable that the drop

in neutrophil counts also reflects change in the sessile and wandering macrophages of the reticuloendothelial system, or some other important unmeasured or unknown factor in host defense. Furthermore, tissue susceptibility to infection may be modified by leukemic infiltration or hemorrhage. The complexity of the situation is confounded even more by the relationship of the infecting organism to the host. Four patients were discovered to be asymptomatic carriers of species of salmonella considered to be "pathogenic." When there was a change in the mature neutrophil count, bacterial infections developed in all these patients due to other organisms and yet in none did any clinical evidence of salmonellosis develop even when their hematologic disease was most active and salmonella were present in the stool.

SUMMARY

1. Fever and infection were studied with comprehensive bacterial, fungal and viral technics in thirty-six consecutive patients with acute leukemia.

2. As a group, these patients were febrile for almost one-half of the total 2,614 patient days. Only two patients had no febrile episodes during the period of observation. Of the ninety-two febrile episodes recorded, fifty-nine appeared to be related to infection. In the remaining thirty-three febrile episodes, bacterial, viral or fungal infection could not be incriminated as a cause for fever.

3. Age and type of leukemia did not influence the frequency of febrile episodes. However, more febrile episodes related to proved or presumptive infection developed in adults than in children.

4. The most common clinical illnesses associated with the proved infections were pharyngitis, pyelonephritis and septicemia. On occasion, relatively avirulent bacteria were capable of causing serious clinical infection. The most frequently encountered organisms were E. coli, M. pyogenes var. aureus, coagulase-positive, and Ps. aeruginosa. Infections due to Ps. aeruginosa were the most difficult to treat.

5. Survival after infection was common, in the absence of bacteremia, if intensive and appropriate antimicrobial therapy was employed. Nevertheless, infection remained a leading cause of death in those patients who died during the study.

6. The majority of the presumptive infections were related to infections of the respiratory tract.

7. In the initial evaluation of the febrile leukemic patient it is to be emphasized that a negative physical examination does not exclude infection as a cause for fever in a patient with acute leukemia.

8. Of the fourteen fevers of undetermined origin which persisted after two weeks of antibiotic therapy, eight eventually terminated in serious bacterial infection. Appropriate management for persistent fever of undetermined origin requires continual reappraisal to insure that a superimposed infection, masked by preceding unexplained fever, has been detected.

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Methods for Analyzing Survival Data, Illustrated by Hodgkin's Disease*

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IN preparation for a clinical study of the therapy of Hodgkin's disease, data in the literature were studied. From these studies, reference curves for survival time (from onset and from either admission, diagnosis or first specific therapy) were derived as a basis for comparison for patients treated with standard local x-ray therapy. Methods of analyzing such data were investigated to find a simple, accurate method for comparing results of therapy. The safety and simplicity of the random number sign test as a method of comparing data was noted. From this study, it was determined that the introduction of antibiotics and alkylating agents have not, as presently used, significantly altered the survival time in Hodgkin's disease. This paper presents the results of these studies.

REFERENCE CURVES

Survival data for Hodgkin's disease fit a logarithmic probability distribution well, as previously noted by Fisher [*1*], Boag [*2*] and Lea [*3*] for carcinomas and by Tivey [*4–6*] for leukemias and many other diseases. By this method, the reference curves can be shown as a straight line on logarithmic probability paper and can be reproduced from just two numbers, the logarithmic mean and standard deviation.

Certain criteria were used in selecting data for reference curves. The series should show evidence that all ages and both sexes were included, that all patients whose biopsies revealed Dorothy Reed cells and who were referred for treatment were included, and that at least five and preferably more points for plotting the survival curve were given. No effort was made to include every series reported; those series which fill the above criteria include enough cases treated at institutions where able radiologists, hematologists and pathologists were known to be active so that the curve would be representative of results obtained with local x-ray therapy at typical research centers.

The curves derived from this data are plotted on logarithmic probability paper in Figure 1. Three months, or 0.25 years, was selected as the unit time period in order to show essentially the entire time course on two cycle logarithmic probability paper.† However, in reproducing these curves for reference, it is suggested that one month be used as the unit for the first cycle of the curve and ten months as the unit for the second cycle of the curve, or that the entire curve be plotted on three cycle logarithmic probability paper.‡ From such plots the predicted percentage survival may be read directly for any time interval and vice versa.

Although I was able to find only two series on which to base a survival time after treatment or diagnosis, namely those of Nice and Stenstrom [*7*], and Bethell et al. [*8*], the reliability of the median value of 20.8 months, determined from these 343 cases, is considerably reinforced by Shimkin and associates' [*9*] median of 21.1 months for 215 cases and Craver's [*10*] median of 18.5 months for 271 males and 26.8 months for 200 females, giving a logarithmic mean or median value of 21.6 months.

The 33.3 months median for the total duration from the three series, Nathanson and Welch [*11*], Shimkin et al. [*9*] and Stout [*12*], totalling 650 cases, is more firmly based numerically. The five-year survival points of both these curves are also reinforced by the data in Tables I and II, but note the extreme scatter of the observed per-

† No. 3128, Codex Book Company, Norwood, Massachusetts.

‡ No. 31,376, Codex Book Company, Norwood, Mass.

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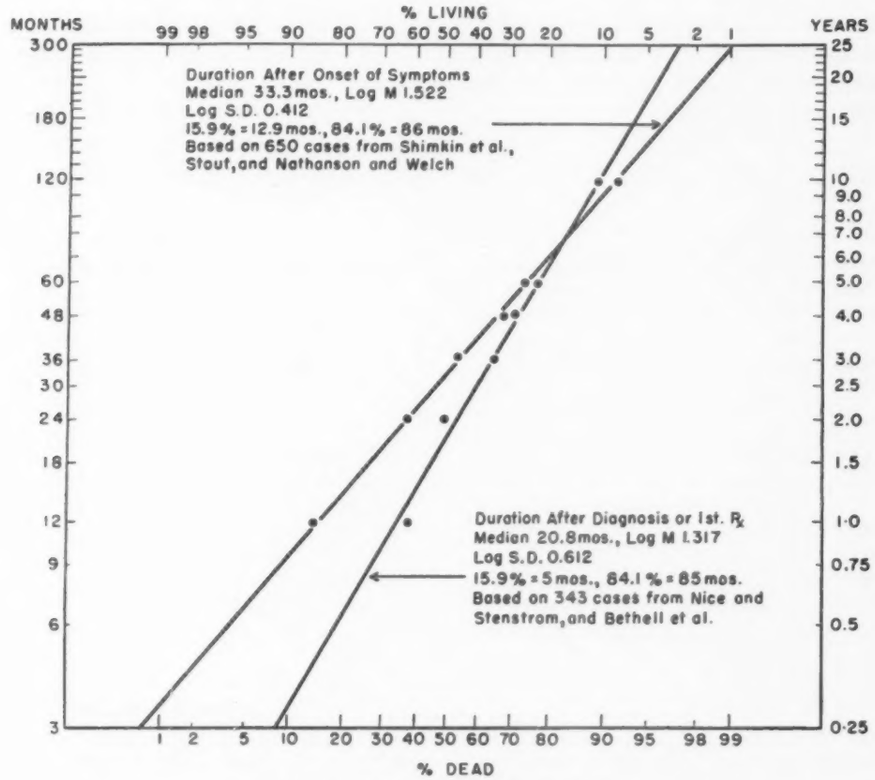

FIG. 1. Survival in Hodgkin's disease.

centages, showing the unreliability of a single point compared with the total curve. Tables I and II undoubtedly contain some duplication, although when several reports were available from the same institution, even by different authors, only the most recent was utilized. The predicted five-year values derived from the logarithmic mean and standard deviation of the reference curves are given in Tables I and II. The observed five-year survival percentages for the combined series used in preparing Figure 1 were 23.1 per cent and 26.1 per cent, respectively. The values predicted from the logarithmic mean and standard deviation of the curves are more reliable, since they are based on the total data. Although, as may be seen in Figure 1, about 35 per cent of the total curve for duration after first treatment and about 15 per cent of the curve for duration after onset is below one year, almost no data on this period were found. A method for reporting data which is ideal for use in such reference curves is that used by Hall and Olson [*21*] for Hodgkin's disease or that which I have used for our leukemic patients [*22*], namely, to report the age, sex, method of treatment, total duration and duration after therapy to the nearest month for each patient. The duration before first specific treatment may be derived by the reader from these figures by subtraction. However, editors are not happy about long tables, and for large series, a satisfactory alternative is to report them as Vera Peters [*23*], (her Table III) and Nice and Stenstrom [*7*] (their Table III) have done, but the three months' and six months' survivals should be included. Space could be saved at the end of the series by grouping the results in five-year intervals after the first ten years. Grouping into two-year intervals between six and ten years would only slightly decrease the reliability. Any method of reporting data which provides ten to twenty well-spaced points on the curve, clearly distinguishes between dead and living patients, and gives the number of patients on which each point is based, may be used.

The arithmetic mean survival is a valueless figure unless the fraction living is given at the time the mean is calculated. The predicted final mean is theoretically determined by taking the antilogarithm of the sum of the logarithm of the median, plus 0.44 times the logarithmic standard deviation, but this value is likely to be

high because of the finite length of human life. It will usually fall between 65 and 70 per cent, therefore it may be read directly from the log probability plot at 67 per cent or, more conservatively, at 65 per cent. The arithmetic mean may be very variable, depending on the chance inclusion or omission of a few long-lived patients. It rarely is available since twenty-five years would have to elapse after the last patient was included in the series before it could be calculated. The predicted means for the two curves in Figure 1 cut off at twenty-five years are 38.5 months after first specific therapy and 50.5 months after onset of symptoms. Reported mean survival times on nearly complete series vary from three to almost five years and the survival times calculated from onset greatly overlap those figured from first therapy. This overlap occurs also in the five-year survival times. The variability of the results is not surprising in view of the great effects of a chance variation in the number of very long lived patients on the mean survival of a series.

TABLE I
FIVE-YEAR SURVIVALS IN HODGKIN'S DISEASE AFTER ADMISSION, FIRST SPECIFIC THERAPY OR DIAGNOSIS

Source of Data	Fraction Living	Per cent
"Cancer at Mid-Century" [13]	126/683	18.45
Boden [14]	29/112	25.9
Craver [10,15]	111/471	23.6
Kaplan and Allen [16]	79/242	32.6
Shimkin et al. [9]	40/215	18.6
Nice and Stenstrom [7]	52/206	25.2
Bethell et al. [8]	23/119	19.3
Total	460/2048	22.46
Predicted from parameters of reference curves	.....	22.6

TABLE II
FIVE-YEAR SURVIVALS AFTER ONSET IN HODGKIN'S DISEASE

Source of Data	Fraction Living	Per cent
Jelliffe and Thomson [17]	50/171	29
Latourette et al. [18]	79/304	26.3
Shimkin et al. [9]	61/230	26.5
Nathanson and Welch [11]	42/184	23
Slaughter and Craver [19]	77/265	29
Stout [12]	49/212	23
Jackson and Parker [20]	51/231	22
Total	409/1597	25.61
Predicted from parameters of reference curves	.....	26.7

USE OF REFERENCE CURVES

The reference curves in Figure 1 may serve as a basis for comparison with any other series. In practice, we have found that the random number sign test to be described is the simplest and safest test for differences. It can be used for

TABLE III
HODGKIN'S DISEASE. RESULTS OF SIGN TEST COMPARISONS

Source of Data	No. of Cases	Total Duration				After First Rx			
		Ties	r	n	p*	Ties	r	n	p*
Wintrobe and Huguley, HN_2 [27]	32	8	11−	24	>0.25	..	....	..	.....
Hall and Olson [21]	66	1	28+	65	>0.25	1	27+	65	0.25
X-ray only	31	1	11+	30	0.25	1	10+	30	0.10
Nitrogen mustard	31	0	15+	31	>0.25	0	15+	31	0.25
Osgood, Seaman and Koler									
Nitrogen mustard and/or TEM	68†	9	27−	59	>0.25	11	25+	57	>0.25
All nitrogen mustard or TEM	131	17	54−	114	>0.25	11	40+	88	>0.25

* p of 0.05 or less would indicate a significant difference.
† Only ten received neither HN_2 nor TEM, and these could have had such therapy.

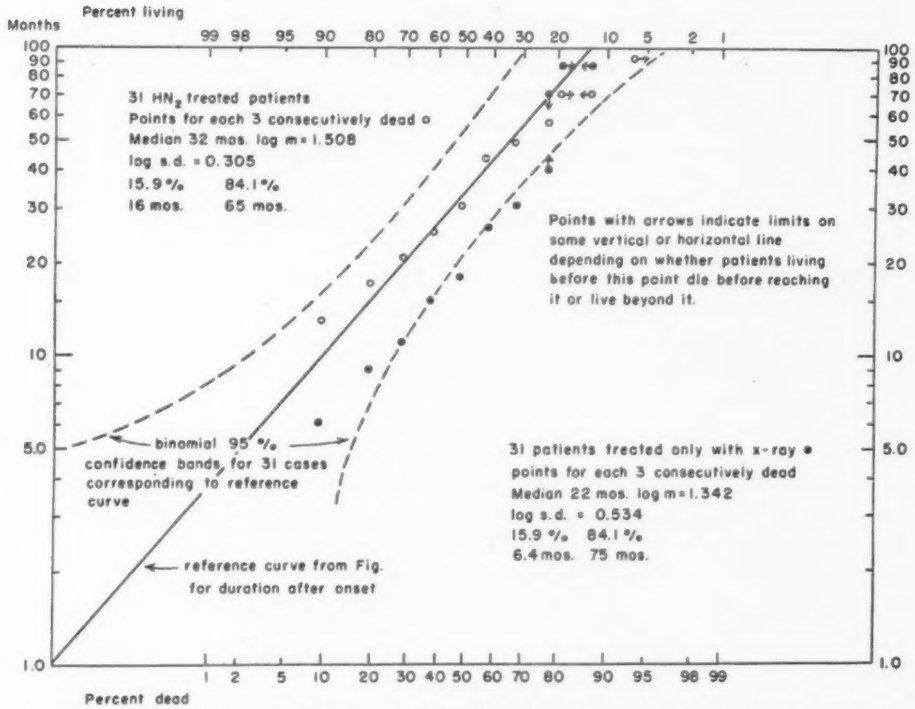
Months
Percent living
99 98 95 90 80 70 60 50 40 30 20 10 5 2 1
100
90
80
70
60
50
40
30
20
10
5.0
1.0
31 HN_2 treated patients
Points for each 3 consecutively dead o
Median 32 mos. log m = 1.508
log s.d. = 0.305
15.9% 84.1%
16 mos. 65 mos.
Points with arrows indicate limits on same vertical or horizontal line depending on whether patients living before this point die before reaching it or live beyond it.
binomial 95% confidence bands for 31 cases corresponding to reference curve
31 patients treated only with x-ray • points for each 3 consecutively dead
Median 22 mos. log m = 1.342
log s.d. = 0.534
15.9% 84.1%
6.4 mos. 75 mos.
reference curve from Fig. for duration after onset
1 2 5 10 20 30 40 50 60 70 80 90 95 98 99
Percent dead

FIG. 2. Hodgkin's disease. Reference curve for duration after onset with 95 per cent confidence bands for thirty-one cases compared with data of Hall and Olsen for thirty-one cases treated with nitrogen mustard and thirty-one patients treated with x-rays.

series with patients still living which would otherwise require the time-consuming maximum likelihood calculations [*3*] before differences between means could be tested. Alternatively and best adapted for large series, individual points of the series may be compared with these reference curves using two standard errors for binomial probability determined as described by Mosteller and Tukey [*24*] using paper such as Codex Book Co. No. 31,298. By connecting a series of such points, a confidence band at any desired level for any number of cases may be drawn on either side of the reference curve. Figure 2 illustrates the data of Hall and Olson [*21*] for thirty-one patients treated with nitrogen mustard with or without x-ray therapy and thirty-one patients treated with x-ray only, compared with the reference curve for duration after onset and its 95 per cent confidence bands computed as described by Mosteller and Tukey [*24*]. Note that both series fall within the 95 per cent confidence limits and that by chance the x-ray treated patients are toward the lower level of confidence for the reference curve rather than the nitrogen mustard series being above it. Conventional statistical methods would require a complete series with variance similar to the reference series before the t-test could be used, and such data are not usually available.

RANDOM NUMBER SIGN TEST ON NITROGEN MUSTARD DATA

While nearly everyone who has utilized the various alkylating agents agrees that they have definite value in controlling systemic symptoms rapidly and in reducing symptoms due to pressure on the trachea, spinal cord or gastrointestinal tract more safely than radiation, there is no statistical evidence that they prolong survival time. Gellhorn and Collins [*25*] first demonstrated this on the basis of random number comparison of sixty-five patients receiving alkylating agents with sixty-five patients treated with local x-ray.

Arthur J. Seaman, Robert D. Koler and I have been treating Hodgkin's disease with alkylating agents for about five years, but the series is as yet too small and has been observed over too short a time to permit reliable maximum likelihood logarithmic probability analysis. The problem of determining whether or not an incomplete series differs from a control series led me to try the sign test as described by Dixon and Massey [*26*] on series paired by random num-

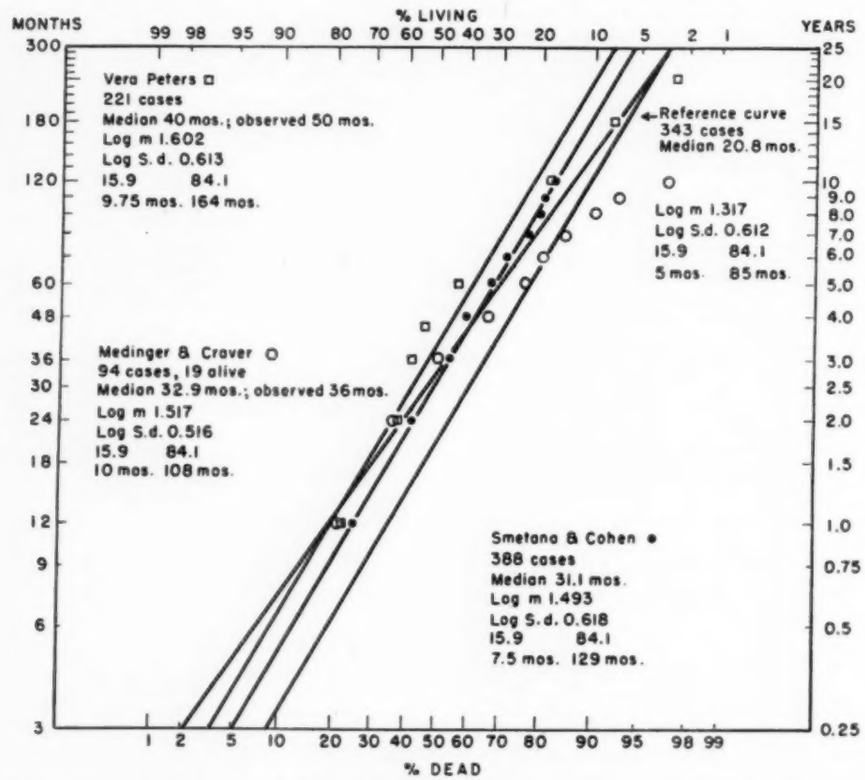

FIG. 3. Hodgkin's disease. Survival after diagnosis or first treatment for three superior series compared with the reference curve.

bers.* This is an extremely simple method of comparing data. While probably not as reliable for large, nearly complete series as the maximum likelihood logarithmic probability analysis, the errors are on the side of conservatism. It can be used safely with a much larger percentage of the patients living, and with series as small as twenty-five, significant differences may be recognized. After many different checks on this sign test, the following method of using it was selected: Replot reference curves such as those given in Figure 1 (at a scale of one month per unit on the first cycle, ten months per unit on the second cycle, and 100 months per unit on the third cycle†) on logarithmic probability paper. Copy on separate cards the predicted survival times at the 1 per cent points, reading the 100th point at the 99.5 per cent line. Mark the points above 300 months, or 25 years, 300 plus to indicate that the patient might be expected to be still living. Draw a series of consecutive random numbers from a table such as is given in Dixon and Massey [*26*], (pages 366 to 370), retaining duplicates, and copy these numbers on the cards in the sequence in which they are drawn. Rearrange these cards in the order of these random numbers.*

Draw a second series of random numbers starting at a different place in the random number table, and assign these in the sequence in which they are drawn to another set of cards for the series being compared to the reference curve which bear the identification, age, sex and duration in months after onset and after first therapy.

* The sign test used in sequence of survival, for which I reported results but from which I drew no conclusions in an article on the natural history of the leukemias [*22*], is not suitable for series that are not nearly complete and may be misleading even in complete series, for if one were to take alternate cases from the same curve and compare them, the second series would by this method appear to be superior to the first.

† If using two cycle paper, change the scale and draw a second line for the second half of the curve.

* Computing the arithmetic mean, five-year survivals, medians, or plotting at the 4 per cent points on log probability paper these values in groups of twenty-five will convince the most skeptical of the hazards of drawing conclusions from such figures derived from small series without statistical tests for significance.

Include the dates of onset and first therapy for all living patients. Arrange these cards also in the sequence of their random numbers; then simply compare the top card in each pile and write down a minus sign if the card of the investigated series showed a shorter survival time than the card of the control series, and a plus sign if the survival time is longer in the investigated series than in the control series. Repeat this for each pair of cards in sequence, marking a zero if the durations differ by less than one month, or if the patient with the shorter survival time is still living, or if both patients compared are living, but tabulate plus or minus values for living patients who have lived longer than dead patients. Count whichever are fewer of the plus or minus signs. This number is r. Subtract the number of zeros (ties) from the total number of cases. The remainder is n. Look in Table A-10, page 417 in Dixon and Massey [*26*] for the p values. Note that r must be the same or *smaller* than the number given in the table under "r" to be significant. Reverse the sign of r to indicate that the investigated series was better or worse than the control series. The sign is of little significance if the p values are 0.25 or more, and a repetition may give a reversed sign. The greater the superiority of a new treatment, the greater will be the proportion of the living patients in that series who will have exceeded the survival time of the paired control card and the fewer will be the patients necessary to demonstrate a significant difference. Theoretically, the same results could be obtained by shuffling each pile of cards, but shuffling is unreliable compared to the use of numbers from a random number table unless it is extremely thoroughly done. The cards may be saved for future comparison of the same or other series.

Interpretation should be: For p values of 0.25 or more, no significant difference has been demonstrated. For p values of 0.05 or less, there is less than one chance in twenty that the difference could have occurred by chance alone. For p values between 0.05 and 0.25, there may be a real difference, but enough data are not available and more time and/or cases will be necessary to justify such a conclusion at the five per cent level of significance.

The advantages of this method of comparison over alternative methods are many. Most of the information available from living patients is utilized. Logarithms of survival times do not have to be used since if time is longer, the logarithm will be larger. Computation is much simpler than for the logarithmic probability maximum likelihood method. It can be used for comparisons within the investigated series of such factors as the influence of age, sex and other variables. Examples of this use are given in "The Results of a 15-year Program of Treatment of the Chronic Leukemias with Titrated, Regularly Spaced Total Body Irradiation with P^{32} or X-ray" to be published in Proceedings of the Sixth Congress, International Society of Hematology by Grune & Stratton. It is well adapted to machine computation when punch card type business machines are available. Theoretically, the ideal method for comparison is to put patients on treatment programs selected only by random numbers. However, this method cuts in half the number of pairs available for comparison. In conditions in which the variability within one treatment program is as great as in Hodgkin's disease, doubling the number of pairs available and being assured that the control is drawn from a population derived from a long term study of a large number of patients treated in the best medical centers probably more than counterbalances this.

The data shown in Table III were derived by the random number sign test and confirm Gellhorn and Collins [*25*] conclusion that nitrogen mustard or TEM, as currently used, has not been demonstrated to prolong survival time significantly in Hodgkin's disease.

Further support for this conclusion and also evidence that modern antibiotics have made little difference in total survival time is gained from the comparison of the first 102 patients treated by Nice and Stenstrom [*7*] before 1935 with the 122 treated since that date, which show no significant difference, and the fact that Shimkin and associates [*9*] observed no significant difference in survival times of their series of patients treated before and after nitrogen mustards were available. Objective criteria such as time at usual work and recreation, decrease in number of hospital days, or transfusions required could well be evaluated when baseline data on these items are published.

Three series of patients were found which did differ significantly from the reference curves. These are shown in Figure 3. The figures of Vera Peters [*23*] are most impressive. The points before five years are taken from her published data on the first 113 patients treated, and those for five years and after from as yet

unpublished data she kindly provided.* The figures of Medinger and Craver [*28*] are also significantly better, and it would seem that the different methods of using ionizing radiation in the treatment of Hodgkin's disease as described in these two reports deserve further trial. The figures of Smetana and Cohen [*29*], coming from the armed services, are a highly selected group. It is difficult to know how significant they are for that reason, but it is noteworthy that these figures almost certainly include all the cases treated by Healy, Amory and Friedman [*30*], who used Peters' method of treatment, and they lend additional support to the superiority of her method of treating Hodgkin's disease.

Some may wonder why a separate analysis has not been made of Hodgkin's disease divided according to the stage or divided according to whether it was classified as Hodgkin's sarcoma, Hodgkin's granuloma or Hodgkin's paragranuloma. The reason these analyses were omitted is that, even in large series, there is such an extraordinary variation in the percentages classified in each of these categories, yet when combined the results of each series are similar. Until there is better agreement on boundary definitions it would seem unsafe to make such separations. Furthermore, the proportion of cases in each category depends on when the patient is first seen during the course of the disease or when the first biopsy is taken. The same patient may be classified in each of the stages or categories at different times. This does not mean that subdivision may not have value as far as individual prognosis is concerned, along with many other factors such as age, sex and associated disease. Actually it would probably be better to subdivide these patients on the basis of sex, because Shimkin [*9*], Craver [*10*] and Jelliffe and Thomson [*17*] have shown a slight but significantly better survival time for females than males with Hodgkin's disease. Dividing the cases, as is done for leukemias, into acute and chronic on the basis of nuclear immaturity and absence of the mature reticulum cell as observed in lymph node imprints will probably also have prognostic value. Finkbeiner, Craver and Diamond [*31*] emphasized the limitations of such subdivisions. These limitations perhaps are explained by "A Unifying Concept of the Etiology of the Leukemias, Lymphomas, and Cancers" [*32*].

Tivey [*6*] has assembled data from which reference curves may be drawn for leukemias, hypertension, myocardial infarction and several carcinomas. See also Boag [*2*], Koler et al. [*33*] and Osgood [*22*] for other reference data.

While the maximum likelihood method of logarithmic probability analysis [*1–6*] still represents the ideal method for analyzing such data for those who are trained in statistical mathematics, the random number sign test as herein described should be a safe alternative for those who do not have access to such skills. It does not require any special mathematical training if the directions herein given are followed.

* The curve of best fit for Dr. Peters' data would pass through 14 per cent living at 300 months or twenty-five years, but because many patients in her series were living below this point, the curve I have shown is actually somewhat more conservative than this. Her observed values were 29 per cent survival of 139 patients at ten years and 25 per cent survival based on sixty-four patients who could have lived over fifteen years.

SUMMARY

Reference logarithmic probability curves based on data in the literature with their parameters are given for survival in Hodgkin's disease after onset and after treatment or diagnosis. Methods of reporting survival data which are better adapted for construction of reference curves are given. The value of the random number sign test and its extreme simplicity as a method of comparison of results of therapy within series and with such reference data is illustrated. The data available do not show any significant improvement in survival time since antibiotics and alkylating agents have been available. Superior results as noted from the literature have been obtained by the use of two different programs of administration of ionizing radiation. These results indicate that it is not enough to specify merely the agent used in therapy. The manner of using that agent may be equally important.

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Cytomegalic Inclusion Disease in Adults*

A Complication of Neoplastic Disease of the Hemopoietic and Reticulohistiocytic Systems

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Two factors combine to make patients suffering from leukemia or from malignant lymphoma peculiarly vulnerable to secondary virus infections. The first is the very nature of viral organisms: obligate intracellular parasites which characteristically produce necrotizing inflammation with relatively little tissue response even in otherwise normal hosts, and which under certain conditions are capable of maintaining for long periods of time a symbiotic state of inactivity in infected tissues. The second is what Dubin [*1*] has called "the poverty of immunologic mechanism" in such patients who with modern usage of chemotherapeutic drugs, steroid hormones and radiation are enabled to survive the effects of their primary disease so long as to be without what little protection their own humoral and cellular defenses provide. Cytomegalic inclusion disease due to generalized infection with salivary gland virus, a rare terminal complication of neoplastic disease of blood and lymphatic tissue, provides a striking example of host-parasite relations altered in this situation to allow activation, release and dissemination of an infectious agent of variable pathogenicity.

GENERAL CONSIDERATIONS

The generic term "salivary gland virus" is applied to related but species-specific sialadenotropic viruses frequently causing dormant infections in man and in laboratory mammals, rarely becoming widely disseminated in other parenchymatous viscera, and producing characteristic gigantism of parasitized cells with distinctly large intranuclear inclusion bodies [*2*]. The term cytomegalic inclusion disease describes the pathognomonic lesion and by authority of common usage implies its viral etiology.

Isolated cytomegalic inclusions are commonly encountered in salivary glands of newborn infants and of young children dying of unrelated causes [*3*]. Less frequently, cytomegalic inclusions are found in association with widespread, multifocal necrotic lesions of parenchymatous viscera and brain of infants dying of an acute febrile disease characterized by pneumonia, hepatic dysfunction or hemopoietic disorder similar to erythroblastosis fetalis [*4*]. Still less frequently, cytomegalic inclusions and associated visceral necrosis are incidental findings in older infants and in children dying of pertussis [*5*]. In both isolated silent and generalized active forms of the disease, salivary gland virus has been recovered and propagated in tissue cultures of human fibroblasts [*6*]. It is likely that most infections are acquired *in utero* and remain quiescent throughout life. Further, it is likely that dissemination is determined by individual susceptibility, depending in young infants upon removal of maternal immunologic defense mechanisms, in older infants and children upon modification of individual defense mechanism by other acute infectious disease. An agent apparently identical with the salivary gland virus has been recovered from nasopharyngeal lymphatic tissue of older children without evidence of related disease [*7*], and it is possible that the virus may remain dormant for years in tissues other than salivary gland.

In adults, generalized cytomegalic inclusion disease occurs only rarely, and there are now only nine cases recorded in the literature of pathology in our language [*8–15*]. There has been no successful attempt to recover a virus but even so the morphology of the lesion is identical with that of known salivary gland virus infection in early life and it is a reasonable assumption that all cytomegalic inclusion disease is of the

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same etiology in different age groups. In some adults the clinical behavior of the disease and the anatomic findings at necropsy are those of uncomplicated generalized viral infection, and death can be explained on this basis. In a larger group of patients there is a background of preexisting chronic infection, malnutrition or hematologic disorder, and the extent and form of cytomegalic lesions are often insufficient to explain death. It is possible that these are individuals in whom dissemination of previously quiescent salivary gland virus is a terminal event made possible by other disease, and in such cases cytomegalic inclusion disease is rarely a cause of death unless there has been extensive necrosis of the adrenal glands. Often obscured as a terminal complication of other disease, it is difficult to assign a characteristic complex of symptoms, physical findings and laboratory data. Except by surgical biopsy, its diagnosis during life is hardly possible.

THE SPECIAL FORMS OF CYTOMEGALIC INCLUSION DISEASE IN ADULTS

The special histologic lesions of cytomegalic inclusion disease in adults are those of a fibrinous pneumonia characterized by miliary necrosis, and a form of multifocal, sometimes confluent necrosis of the adrenal glands.

At the necropsy table the lungs appear to be shot through with multiple firm, rubbery nodules that are 3 to 10 mm. in diameter, generally sharply defined and scattered, but sometimes in congeries or confluent aggregates. (Fig. 1.) There is no orientation to bronchioles or blood vessels, although as a rule most are in the periphery of the lung and often subpleural. They may be superimposed upon any combination of bronchopneumonia, congestive hyperemia, edema or other non-specific gross lesion. On surfaces made by cutting, the nodules are everted, dark red-purple, with grey or yellow umbilicated central areas of necrosis. They are often surrounded by a hyperemic blush or are set in areas of fresh hemorrhage.

The microscopic appearance of this form of pneumonia depends upon the age of the lesion. Early, there are discrete foci of hemorrhagic necrosis in which large aggregates of alveolar spaces, filled with erythrocytes and strands of fibrin, adult granulocytes, histiocytes and cell debris, are lined by swollen alveolar cells, many of which have one or more grotesque giant nuclei with inclusion bodies. (Fig. 2.) These bodies are

FIG. 1. Case 1. Pneumonia due to cytomegalic inclusion disease in a patient with Hodgkin's sarcoma. Subpleural localization of discrete miliary nodules of hemorrhagic inflammation and necrosis in a partially atelectatic segment of lung. Hematoxylin and eosin stain, 72 mm. microtessar lens, × 15.

round, oval or kidney shaped, and but for a thin clear area intervening between the thick nuclear membrane very nearly fill the entire large nucleus. (Fig. 3.) They are well characterized by the term "owl-eyed," as widely used in the literature of this disease, and their tinctorial properties vary from basophilia to metachromasia. Although of different morphologic characteristics, their histochemical properties are those of other viral inclusion bodies and in no way do they resemble the oxyphilic nucleoli of Reed-Sternberg cells or other structures of neoplastic reticulohistiocytes. Parasitized giant cells frequently appear to be budding from swollen littoral cells and may be free in pools of blood and fibrinous exudate in alveolar spaces. (Fig. 4.) Hyaline alveolar membranes may be prominent. In some cases of mixed infection tiny mycotic organisms of Pneumocystis carinii or Toxoplasma gondii have been found free or in the cytoplasm of littoral cells and histiocytes

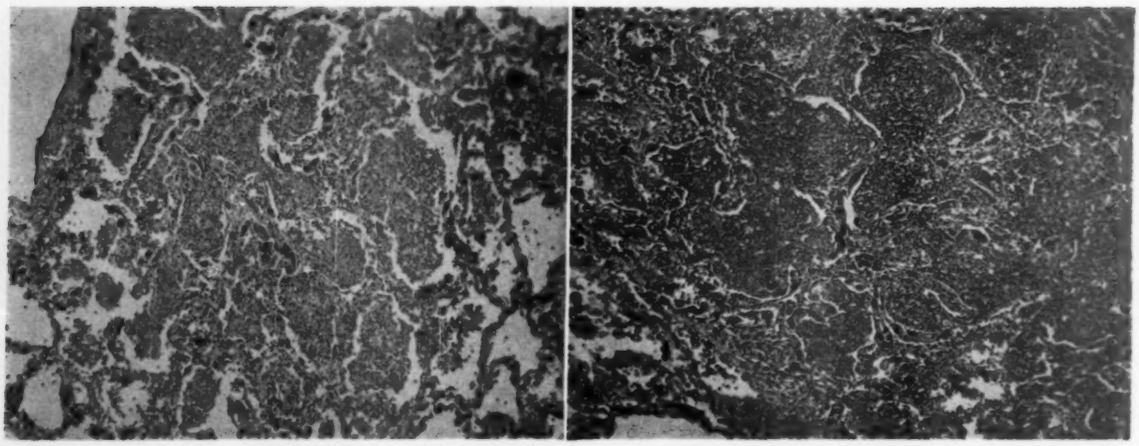

FIG. 2. Case 1. Pneumonia due to cytomegalic inclusion disease in a patient with Hodgkin's sarcoma. Miliary nodules are composed of aggregated alveolar spaces filled with sanguinofibrinous exudate and lined by giant alveolar cells with characteristic intranuclear inclusion bodies. At this magnification the parasitized giant cells may simulate Reed-Sternberg cells because the nuclei are hyperchromatic and their inclusions may be mistaken for nucleoli. Hematoxylin and eosin stain, 16 mm. apochromatic objective lens, × 125.

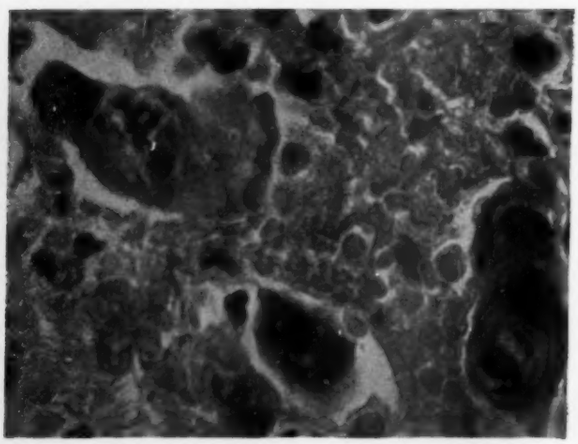

FIG. 3. Case 1. Pneumonia due to cytomegalic inclusion disease in a patient with Hodgkin's sarcoma. Parasitized alveolar littoral cells at the lower left have giant nuclei with huge intranuclear inclusions that very nearly fill their whole structure. The intra-alveolar exudate is composed of erythrocytes and a scanty admixture of leukocytes, with two free giant cells at the bottom of the photograph and at the upper left. The last of these has intracytoplasmic bits of phagocytized nuclear debris, as well as a large intranuclear inclusion body. Hematoxylin and eosin stain, 3 mm. apochromatic oil objective lens, × 1200.

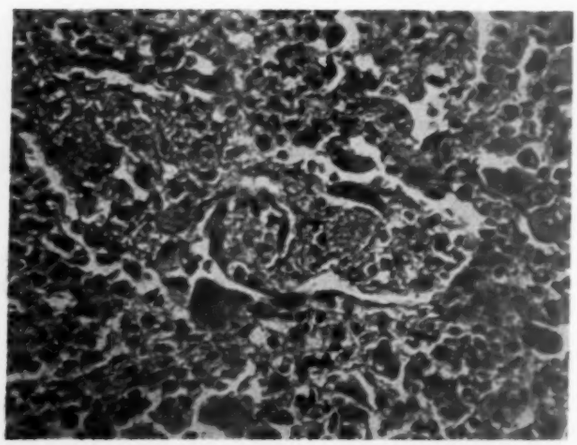

FIG. 4. Case 1. Pneumonia due to cytomegalic inclusion disease in a patient with Hodgkin's sarcoma. An alveolar space is filled with strands of fibrin and proteinaceous coagulum in which are suspended erythrocytes, a few lymphocytes and free giant cells. The septums are thickened and infiltrated with a mixture of leukocytes. Hematoxylin and eosin stain, 8 mm. apochromatic objective lens, × 250.

[*14,15*]. In the late lesions there are attempts at organization of the alveolar exudate, development of a chronic inflammatory cellular response and interstitial fibrosis.

In the adrenal glands there is essentially the same process modified by structural peculiarities of the tissue and, in the special situation with which this report deals, partially obscured by focal lesions of malignant lymphoma, leukemic infiltration or metaplasia, and by cortical changes consequent to steroid therapy. As a rule there are punctate hemorrhagic lesions of the medulla but, if extensive, there may be large confluent, yellow-grey to red, soft areas of necrosis of the medulla and inner zones of the cortex. Microscopically there may be either circumscribed or diffuse necrosis of all elements. (Fig. 5.) Intermixed in the debris in areas of necrosis are erythrocytes and large numbers of karyorrhectic granulocytes, while around the periphery many surviving parenchymal cord

FIG. 5. Case II. Focal adrenal necrosis due to cytomegalic inclusion disease in a patient with granulocytic leukemia. In the center of the photograph is an area of necrosis, around the periphery of which are scattered parasitized giant cells. At "9 o'clock" there is a cell with a large intranuclear inclusion body. Neoplastic extramedullary myelopoiesis obscures the normal architectural pattern of the gland. Hematoxylin and eosin stain, 16 mm. apochromatic objective lens, × 125.

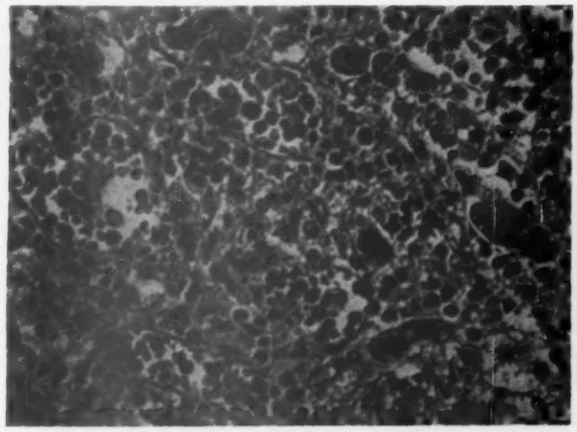

FIG. 6. Case II. The lesion of cytomegalic inclusion disease in the adrenal gland in a patient with granulocytic leukemia. Parasitized giant cells with large intranuclear inclusions lie in and alongside sinusoids filled with leukemic cells. At "11 o'clock" there is a very distinct inclusion body. Hematoxylin and eosin stain, 8 mm. apochromatic objective lens, × 250.

cells possess the characteristic giant nuclei and inclusion bodies. (Fig. 6.)

THE HEMOPOIETIC AND RETICULOHISTIOCYTIC SYSTEMS IN CYTOMEGALIC INCLUSION DISEASE

There is a close and as yet poorly understood relation between cytomegalic inclusion disease in the adult and disease of the hemopoietic and reticulohistiocytic systems. Generalized salivary gland virus infection provokes a florid lymphocytic inflammatory tissue response, the pattern of which may superficially resemble a form of lymphomatous neoplasm; in some cases it is possible to determine the true nature of the lesion only after complete necropsy study [*13*]. Further, extramedullary myelopoiesis in secondary hemopoietic organs, a constant feature of the infantile form of the disease, has been described in many but not in all cases of cytomegalic inclusion disease in adults and may involve primitive hemopoietic cells of such a nature as to make exclusion of granulocytic leukemia very difficult. This is of especial interest because, on the other hand, dissemination of salivary gland virus may be a terminal event in cases of true neoplastc diseases of lymphatic tissue and blood. In four of the nine reported cases cytomegalic inclusion disease has been superimposed on a severe primary hematologic disorder characterized by marrow failure and panhemocytopenia: primary refractory anemia, an ill-defined form of aleukemic myelosis and forms of malignant lymphoma. (Table I.) It is not likely that there is an etiologic relation between salivary gland virus infection and diseases of such diverse morphology and behavior, but rather that in these diseases there is a special situation favorable to the dissemination and development of the infection. In the past year and a half we in this laboratory have encountered the disease at necropsy in two cases of malignant lymphoma and in one case of granulocytic leukemia, all well documented by adequate clinical study.

REPORT OF THREE CASES

CASE I. *Cytomegalic Inclusion Disease in Hodgkin's Sarcoma.* A thirty-one year old Caucasian automobile salesman was admitted to this hospital for the ninth time on January 1, 1956. There was a history of Hodgkin's sarcoma diagnosed by biopsy of an enlarged mass of mesenteric lymph nodes three and one-half years earlier. He had first been treated by radiation with good response; since that time there had been eight admissions for weakness, fever, anemia and malaise, and each time he had responded well to courses of radiation. One year before death and then again six months before death he had received courses of treatment with nitrogen mustard, with good palliative effect on the course of his disease. Severe hemolytic anemia had developed; throughout his illness he had received many transfusions of whole blood but without lasting effect. Three months before death he had recurrence of fever and night sweats for which he consulted an eminent Texas osteopath who gave him iron, liver and a preparation called "Cancer 100,"

TABLE 1

CASES OF CYTOMEGALIC INCLUSION DISEASE IN ADULTS WITH PRIMARY DISEASES OF THE HEMOPOIETIC AND RETICULOHISTIOCYTIC SYSTEMS

Author	Primary Disease	Chemotherapy	Findings at Necropsy				
			Lesions of Primary Disease	Lesions of Cytomegalic Inclusion Disease			Other Significant Lesion
				Lungs	Adrenals	Other Organs	
WYATT, J. P., HEMSATH, F. A. and SOASH, M. D. [10]	Primary refractory anemia of 7 years duration; panhemocytopenia	None	Hypoplasia of marrow with early fibrosis	+	+	Liver, pancreas, duodenum	Hemosiderosis
REINHARD, E. H., GOOD, J. T. and MARTIN, E. [11]	Acute lymphocytic leukemia, subleukemic variety (leukosarcoma) of 8 months' duration; anemia, leukopenia	4-aminopteroylaspartic acid	Hypoplasia of marrow; hemosiderosis of marrow, spleen and lymph nodes; no residual leukosarcoma	+	..	Liver, spleen	Hemosiderosis; lipoid pneumonia
HEMSATH, F. A. and PINKERTON, H. [15]	Myeloid metaplasia of the spleen, with splenomegaly, anemia, leukopenia and thrombocytopenia of unknown duration	None	Marked hyperplasia and anaplasia of marrow; myeloid metaplasia of spleen	+	+	Liver, spleen, duodenum	Toxoplasmosis
HAMPERL, H. [14]	Hodgkin's sarcoma of unstated duration	Not stated	Hodgkin's sarcoma	+	..	.....	Emaciation
PEACE R. J.	Hodgkin's sarcoma of 3½ years duration; hemolytic anemia, leukopenia	Nitrogen mustard	Marked hypoplasia of marrow; Hodgkin's sarcoma involving spleen and abdominal lymph nodes	+	..	.....	Cholangiolitic viral hepatitis; emaciation
	Subacute granulocytic leukemia of 6 months duration; normocytic myelophthisic anemia	1,4-dimethanesulfonyloxybutane, 6-mercaptopurine, desacetylmethylcolchicine	Subacute granulocytic leukemia with neoplastic replacement of marrow, spleen, lymph nodes	..	+	Liver	Emaciation
	Generalized lymphoblastic lymphosarcoma of 6 years duration; variable anemia, leukopenia	Nitrogen mustard	Generalized lymphoblastic lymphosarcoma	..	+	.....	.....

all in vain. One week before the final admission rapidly deepening jaundice, dark urine and mental confusion developed.

On admission his blood pressure was 95/45 mm. Hg; his pulse, 140 per minute; his temperature, 100.8°F.; and his respiratory rate, 20 per minute. Although well developed, he was emaciated, acutely ill, weak and dehydrated. There was a herpetic ulcer of the tongue, fine crepitant rales over the bases of both lungs, and sinus tachycardia. The liver extended 1 cm. below the costal margin and had a sharp, slightly tender edge. The spleen was not palpable. There was bilateral inguinal lymph node enlargement. The skin of the extremities was covered with petechiae. The hemoglobin was 5.2 gm. per cent; the hematocrit, 19 per cent; the erythrocyte sedimentation rate, 73 mm. in the first hour; and the reticulocyte count was 3.1 per cent. The leukocyte count was 5,100 per cu. mm. with a normal differential spread and adequate platelets. Coombs' test was negative. Hyperchloremia and hyponatremia were noted. The urine contained albumin and acetone as well as bilirubin crystals. The total serum protein was 3.9 gm. per cent, with an albumin-globulin ratio of 1:1. The total serum bilirubin was 12.6 mg. per cent, of which 7.3 mg. were indirect reacting. The serum alkaline phosphatase was 10.5 Bodansky units; thymol turbidity, 1 unit; and the prothrombin time, 30 per cent of the normal. The hemoglobin consistently remained below 7 gm. despite repeated transfusions. A roentgenogram of the chest showed only slight elevation of the diaphragmatic leaves.

Because it was thought that there was compression of the extrahepatic biliary tract by a lymphomatous mass, radiation was given over the right upper quadrant of the abdomen. In addition, the patient received lyophilized adrenocorticotropic hormone for ten days, and then prednisone. On the sixteenth

day he became comatose and with the development of extensive ecchymoses, petechiae and melena he died quietly on the eighteenth hospital day.

At necropsy there was malignant lymphomatous involvement (Hodgkin's sarcoma) of the spleen and abdominal lymph nodes, multiple splenic infarcts, cholangiolitic viral hepatitis, diffuse superficial ulceration of esophageal and gastric mucosa, and diffuse interstitial hemorrhage into the papillae and pelvic mucosa of both kidneys. The left lung weighed 170 gm.; the right lung 350 gm. There were multiple small nodules the size of bird shot (3 to 4 mm. in diameter) scattered throughout. These nodules were firm, discrete and had dark red-purple, faintly umbilicated cut surfaces. Dependent portions of the lower lobes were boggy, while the upper lobes were light and crepitant. The surfaces made by cutting dependent portions were wet, reddish and markedly congested. Both adrenal glands were somewhat enlarged and softer than normal. The cortices were homogenous, dusky grey, firm and glistening. There was no gross lesion of the medulla.

Microscopic examination revealed extensive neoplastic infiltrate and replacement of the substance of the spleen and abdominal lymph nodes by masses of neoplastic lymphoid tissue with many bizarre, multinucleated reticulohistiocytic elements. Sections of lung showed a well-marked cytomegalic inclusion body pneumonitis without associated Pneumocystis carinii infection. The shot-like nodules proved to be aggregates of alveoli filled with blood, fibrin, histiocytes and cell debris; all were lined by huge distorted cells containing giant nuclei with faintly oxyphilic or metachromatic inclusion bodies. There was some paravascular fibrosis but no pulmonary lesion of Hodgkin's granuloma. Cytomegalic inclusions were not found in any other organ. The esophageal ulcer had neither inclusions nor demonstrable microorganisms and was attributed to the effects of chemotherapy.

Case II. *Cytomegalic Inclusion Disease in Subacute Granulocytic Leukemia.* A twenty-nine year old Caucasian carpenter was admitted to this hospital on April 4, 1956, in "blast" crisis of granulocytic leukemia of six months' known duration. He had been treated unsuccessfully with 1,4-dimethanesulfonyloxybutane, but with repeated courses of 6-mercaptopurine and transfusions of whole blood his disease had been well controlled.

The blood pressure was 140/60 mm. Hg; the temperature, 100.6°F.; the respiratory rate, 22 per minute; and the pulse rate, 90 per minute. There was slight dyspnea, marked protuberance of the abdomen and muscle wastage of the extremities. The heart was enlarged and there was a blowing holosystolic hemodynamic murmur over the entire precordium. The liver and spleen were markedly enlarged and there was generalized lymph node enlargement and peripheral edema.

The total leukocyte count was 190,000 per cu. mm. with 8 per cent adult neutrophil granulocytes, 1 per cent metamyelocytes, 5 per cent myelocytes, 33 per cent promyelocytes and 53 per cent hemocytoblasts. Platelets were numerous. The hemoglobin was 5.9 gm. per cent; the hematocrit, 19. The serum uric acid was 12.7 mg. per cent. Roentgenograms of the chest showed hilar lymph node enlargement and increased linear markings throughout both lung fields with a poorly defined infiltrate in the lower lobe of the right lung. Subsequent roentgenograms showed infiltration into the base of the left lung.

Because of the continued febrile course, dosage of 6-mercaptopurine was increased on three occasions, each time without effect. Peripheral edema became progressively more marked and, because the patient had failed to respond to either mercaptopurine or dimethanesulfonyloxybutane, a course of therapy with desacetylmethylcolchicine (a synthetic antimitotic derivative of colchicine) was begun. His condition continued without change until the morning of the fortieth hospital day, when he complained of drowsiness; late that afternoon he suddenly collapsed and died with electrocardiographically documented ventricular fibrillation.

At necropsy there was massive splenomegaly and hepatomegaly due to neoplastic infiltration and replacement by leukemic cells. The lungs were edematous and had congestive hyperemia with multiple old and recent thrombi of pulmonary vessels. There was ulcerative esophagitis. The left adrenal gland weighed 20 gm.; the right adrenal gland weighed 23 gm. Both were dark reddish brown, mottled and firm. The cortices were light tan and thickened; the medullae were greyish white and of almost gelatinous consistence.

Microscopic examination of sections of marrow, spleen, lymph nodes, thymolymphatic tissues and parenchymatous viscera showed neoplastic extramedullary myelopoiesis and replacement by the cellular infiltrate of subacute granulocytic (myeloblastic) leukemia. Sections of adrenal gland showed a remarkable mixture of lesions which combined to almost completely destroy the cortical element. In addition to massive neoplastic infiltration there was severe tubular degeneration and large areas of frank necrosis of both medulla and cortex. Around areas of necrosis many of the parenchymal cells were quite large, had giant nuclei with prominent intranuclear inclusions that were oxyphilic and pathognomonic of salivary gland virus disease. Similar inclusions were found in the liver although in that organ the lesions were less prominent. There was no evidence of inclusion bodies or microorganisms in the area of esophageal ulceration.

Because two examples of such a rare disease were encountered in a small laboratory in a very short period of time and in cases of essentially similar nature it was decided to review all our

cases in which a hematologic disorder was studied at necropsy. Files were available for a period of eight years, and approximately two hundred cases, including examples of malignant lymphoma, leukemias, hemolytic anemias, aplastic anemias, myelofibrosis and other hematologic disorders were reviewed. One case was encountered in which the microscopic lesion of cytomegalic inclusion disease was minimal and had originally been overlooked.

CASE III. *Cytomegalic Inclusion Disease in Generalized Lymphoblastic Lymphosarcoma.* A thirty-six year old Negro farmer was admitted to this hospital on April 8, 1955, giving a history of malignant lymphoma treated over a period of five years by repeated courses of nitrogen mustard and radiation. He had originally sought medical aid because of pain in the lower part of the abdomen, swelling of the groins and of the armpits, night sweats and edema of the ankles, all of approximately one year's duration. At that time he was thin, asthenic and had, in addition to generalized lymph node enlargement, multiple intra-abdominal masses just above the symphysis pubis. The erythrocyte count was 3 million per cu. mm., with a hemoglobin of 11 gm. per cent. The total leukocyte count was 4,000 per cu. mm., with 42 per cent adult neutrophil granulocytes, 52 per cent lymphocytes and 6 per cent monocytes; the bone marrow was hypocellular. On biopsy of an axillary lymph node, the diagnosis of Hodgkin's disease was submitted. He was treated with massive irradiation to the abdomen and with whole blood transfusions, with good results. Following this he was given two doses of tetraethylmelamine and then nitrogen mustard with maximum benefit. He returned again after periods of one year and of two years; on both occasions he was given intensive nitrogen mustard treatment and repeated transfusions of whole blood. On the final admission in the fifth year of his disease he related symptoms and showed signs of recently accelerated progressive lymphomatous involvement of peripheral and abdominal lymph nodes, abdominal viscera, skin and central nervous system. He was given 10 mg. of nitrogen mustard daily for three days without effect. Persistent massive ascites, abdominal pain and daily temperature elevations to 102° to 103°F., semicoma, tachycardia and dyspnea developed, and with such progressive deterioration he died on the eighteenth hospital day.

At necropsy there was widely disseminated lymphoblastic lymphosarcoma involving the heart and pericardium, lungs, all the lymph nodes, all segments of the gastrointestinal tract, liver, pancreas, kidneys, urinary bladder, testes, dura and leptomeninges, pituitary and adrenal glands, retroperitoneal and mesenteric fat, bones and skin. Of especial interest was a large mass of neoplastic tissue that filled most of the true pelvis. Other findings were those of ascites and acute ulcerative esophagitis.

The left lung weighed 580 gm. and the right lung weighed 550 gm. Both were soft and crepitant in most places but soggy in the dependent portions. There was a vague nodularity and the surfaces made by cutting were bloody, moist and mottled red. There were a few small peribronchial nodules that were up to 8 mm. in diameter. Both adrenal glands were normal in size, shape and position. They were encased in dense, white, rubbery, smooth, fleshy, lymphomatous neoplastic tissue. The cortices were brown and the medullae greyish white.

Microscopic examination revealed tiny foci of minimal necrosis of the adrenal medulla and several parenchymal cells containing pathognomic intranuclear inclusions of salivary gland virus infection. Inclusions were not found in any other tissue. Again, an especially careful but fruitless search was made for intranuclear inclusions in the vicinity of the ulcerative esophagitis.

COMMENTS

On the face of it, generalized infection with salivary gland virus may complicate neoplastic disease of blood or lymphoid tissue in the same manner as infections with cryptococcosis and histoplasmosis, but there can be only speculation as to the different pathogenetic mechanisms involved. Although unlikely, it is possible that those with dormant infections are carriers and that in the special situation reported here, transfusion of whole blood from such carriers may introduce the salivary gland virus into individuals with maximum susceptibility who may also be without previous experience with the disease and incapable of effectively reacting against it. The disease is undoubtedly present in the form of latent infection in many persons, and so may become disseminated in the course of other disease of the reticulohistiocytic system. Of the cases included in this report, the lesions of cytomegalic inclusion disease were best developed in instances in which there had been extensive and nearly complete replacement of the normal structure of the spleen and lymph nodes by neoplastic tissue, suggesting a parallel of observations that in immunized experimental animals, splenectomy is followed by decrease in concentration of agglutinins and protective antibodies [*16*]. This is not to say that the spleen provides some intrinsic protective factor but rather that decrease in effective mass of reticulohistiocytic tissue, due to whatever cause, might permit spread of a virus infection formerly restricted to only one tissue.

This is a situation ideally suited to both dissemination of a newly acquired infection and to exaltation and release of a previously quiescent infection. Not only is there impairment of mechanisms of host resistance as a part of the basic disease but these mechanisms are further modified and the response to injury hampered by treatment of that disease. Steroid hormones are known to lower resistance to pathogenic microorganisms and prevent adequate inflammatory response even in a normal host. Further, in this application the use of chemotherapeutic drugs and radiation is ultimately only an attempt at crudely controlled destruction of hemopoietic and reticulohistiocytic tissue by competitive interference in cellular enzyme systems, direct cytotoxic action or modification of growth patterns, all of which modify growth of the viral organism and depress reactivity of the infected tissue [*13,16*]. In all three cases reported here the patients had a very significant feature in common: severe anemia related to a malignant neoplastic disease of blood or lymphatic tissue treated by repeated and intensive courses of chemotherapy. This calls to mind another reported case in which cytomegalic inclusion disease complicated malignant lymphoma treated intensively with a chemotherapeutic agent [*11*], and the observation that spontaneous cytomegalic inclusion disease occurs in guinea pigs treated with an antimetabolite (4-aminopteroylglutamic acid) used in leukemia therapy [*4*].

Even in this situation antibiotics permit control of most intercurrent bacterial infections until eventually the disease is carried to the point where the patient, in a state of near-anergy and supported chiefly by repeated blood transfusions, is exposed without defense to the whole group of viruses and fungi, even those of minimal or variable pathogenicity.

Although long known, cytomegalic inclusion disease is still not likely recognized and is often overlooked even by competent pathologists [*15*]. It is suggested that perhaps in some cases of leukemia or Hodgkin's disease in adults with secondary dissemination of salivary gland virus, parasitized giant cells might have been confused with neoplastic reticulohistiocytes or hemocytoblasts with large nucleoli. With increased awareness of the disease and continued use of present day therapy it is likely that terminal viral and mycotic infections in neoplastic diseases of blood and lymphatic tissue will be increasingly frequent and that cytomegalic inclusion disease in adults will be encountered and recognized more frequently.

SUMMARY

Cytomegalic inclusion disease in adults is discussed as a rare terminal complication due to dissemination of salivary gland virus in neoplastic diseases of the hemopoietic and reticulohistiocytic systems. Three additional cases are reported. The possible pathogenesis of the disease is discussed, giving emphasis to the role of chemotherapy.

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Short-term Penicillin and Dihydrostreptomycin Therapy of Streptococcal Endocarditis*

Results of the Treatment of Thirty-five Patients

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IT is well established that penicillin is the most effective drug in the treatment of bacterial endocarditis caused by the viridans streptococci and the other streptococci which are relatively sensitive to its action *in vitro*. Drugs such as sulfonamides, chloramphenicol and the tetracyclines, which may suppress growth of the infecting microorganisms over long periods of time, have proved valueless or distinctly inferior to penicillin. This is apparently due to the fact that the host mechanisms of defense are unable to eliminate those bacteria which are suppressed but not sufficiently reduced in numbers by the antibiotic. That the host defenses are relatively ineffective in bacterial endocarditis is further emphasized by the very rare occurrence of spontaneous recovery from this disease.

Although penicillin has proved highly effective, presumably because of its greater bactericidal effect, it has been demonstrated that successful therapy depends on the administration of adequate amounts of the drug over a sufficiently long period of time. Thus far, no treatment regimen has been devised which results in bacteriologic cure in all cases. It has been the general experience [*1*] that it is necessary to administer penicillin in moderately high dosage for long periods of time to avoid failure to cure the infection with the first course of treatment. If treatment with moderately high doses is discontinued at twenty-eight days a significant number of patients, about 10 to 15 per cent, have a relapse. For example, in the British Medical Research Council studies [*2*], of a total of 129 patients who received 500,000 units of penicillin daily for twenty-eight days, eighteen (15 per cent) relapsed or died with uncontrolled infection. Of the eighteen failures four were infected with highly penicillin-resistant organisms. If these are excluded the number of failures is fourteen (11 per cent). To achieve a relapse rate approaching zero by the use of penicillin alone, it has seemed necessary to prolong therapy to six to eight weeks [*3,4*].

Following the introduction of streptomycin several studies suggested the possibility that streptomycin and penicillin acting together might have a more truly bactericidal action on streptococci than either drug alone. In 1948 Spicer and Blitz [*5*] observed the survival of significant numbers of viridans streptococci and enterococci after exposure to concentrations of penicillin well above the "minimal inhibitory concentration" for the strain. They also found that the surviving members of the bacterial population were often killed by low concentrations of streptomycin. In 1949 the effectiveness of long-term therapy with concurrent penicillin and streptomycin was demonstrated in a sizable group of patients with enterococcal endocarditis [*6,7*], and in a case of penicillin-susceptible streptococcal endocarditis which had been especially resistant to penicillin therapy over the course of more than two years [*8*].

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The encouraging results with concurrent administration of penicillin and streptomycin led to initiation of the present study in 1949. The purpose was to determine whether or not the combined use of penicillin and streptomycin (or dihydrostreptomycin) would prove to be more effective antimicrobial therapy than penicillin alone in penicillin-susceptible streptococcal endocarditis. It was decided to employ both drugs in moderate dosage over a short period of fourteen days, a duration of treatment which, if penicillin alone were used, would result in a high incidence of relapse.* It was believed that the demonstration that an experimental short-term combined regimen resulted in a low incidence of relapse would be additional evidence for the increased bactericidal effect of this drug combination on these organisms, as well as possibly affording a shorter therapeutic regimen for the disease.

Since this study was begun further evidence has been presented for the synergistic action of penicillin and streptomycin on various nonhemolytic streptococci *in vitro* [*11,12*]; in the treatment of enterococcal endocarditis [*13*]; and in the treatment of penicillin-susceptible streptococcal endocarditis [*14–17*]. It has been found that the bactericidal effect of penicillin could be strikingly enhanced by streptomycin in some strains of viridans streptococci; in other strains it was not demonstrated [*12*]. In no instance, however, has addition of streptomycin to penicillin resulted in diminished bactericidal action (antagonism) on these organisms *in vitro*. There can be little doubt that this combination of drugs is highly bactericidal *in vitro* for many strains of enterococci and viridans streptococci, and that it is the only predictably successful drug therapy now available for enterococcal endocarditis. The evidence for greater effectiveness than penicillin alone in penicillin-susceptible streptococcal endocarditis has been very suggestive, although more difficult to evaluate because of the generally good results obtainable with penicillin alone. Geraci [*15,16*] has reported a total of forty-six patients with penicillin-sensitive streptococcal endocarditis given short-term penicillin and streptomycin therapy without a single recorded instance of bacteriologic failure. Hall and his associates [*17*] have reported fifteen patients similarly treated, also with no instance of proved bacteriologic failure; one patient was re-treated because of persistent fever although bacteriologic relapse could not be established.

It is the purpose of the present paper to report our experience with the short-term concurrent penicillin and dihydrostreptomycin therapy of thirty-five patients with endocarditis caused by penicillin-susceptible streptococci.

* In this regard the results of King et al. [*9*] and of Hamburger and Stein [*10*] with massive short-term penicillin therapy seem to conflict In the former study the relapse incidence was 88 per cent whereas in the latter it was only 17 per cent. Almost identical total daily doses of penicillin were employed but it may be significant that in the former series caronamide was also given and much higher penicillin levels were achieved, perhaps above the optimal range for the organisms concerned, as was suggested by the authors themselves [*9*]. Furthermore, in the former study treatment was of only ten days' duration whereas in the latter it was continued for fourteen days.

PLAN OF STUDY AND CLINICAL SERIES

All thirty-five patients in this study were treated at the New York Hospital between 1949 and 1955. Pertinent clinical data are summarized in Table 1. All patients had clinical evidence of valvular or congenital heart disease and all were febrile at the time treatment was instituted. Other manifestations of bacterial endocarditis were present in the majority. Only those cases are included in which bacteriologic confirmation of the diagnosis was obtained, and in which the infecting organism was relatively sensitive to penicillin *in vitro*. In thirty-two patients the infecting organism was recovered from two or more blood cultures prior to treatment; in three patients only one positive culture was obtained. The series represents a selected group in the following additional respects: In the early part of the study some patients were not included because of the fact that this was a new and controversial type of treatment. A few patients were excluded also in whom treatment was extended beyond the planned two-week period, in some instances as prophylaxis pending cardiac surgery. In no instance was therapy extended or altered because of the persistence or return of bacteremia during treatment.

Thirty-three of the streptococcal strains isolated from the blood of these patients have been classed simply as viridans streptococci. A group H streptococcus was recovered from two patients and a "microaerophilic" streptococcus from another. Thirty-three strains were sensitive to penicillin in concentrations of 0.4 unit/ml. or less as measured by a standard bacteriostatic tube dilution test; twenty-six were inhibited

TABLE I

SHORT-TERM PENICILLIN AND DEHYDROSTREPTOMYCIN THERAPY OF STREPTOCOCCAL ENDOCARDITIS. CLINICAL DATA ON 35 PATIENTS

Case No.	Age (yr.)	Sex	Duration of Symptoms before Present Treatment Started	Infecting Micro-organism	Sensitivity		Type of Heart Disease	Associated Conditions	Antimicrobial Therapy (daily dosage)		Days of Treatment	Other Treatment in Follow-up Period	Results of Treatment	Follow-up	Comment
					Penicillin (U/nl.)	Streptomycin (μg./ml.)			Penicillin (million units)	Dihydrostreptomycin (gm.)					
1. Si	59	M	2–3 mo.	Strep. viridans	0.08	8.0	Congenital (interventricular septal defect)	.....	1.6	2.0	12	None	Bacteriologic cure	2 yr.	Died of unknown cause 2 years after completion of treatment
2. St	33	M	2 wk.	Strep. viridans	0.31	2.0	Congenital (pulmonic stenosis)	.....	2.4	2.0	14	None	Bacteriologic cure	6½ yr.	.....
3. Te	36	F	6 wk.	Strep. viridans	0.05	1.6	Rheumatic (MI, MS, AI, AS)	Chronic pyelonephritis; aneurysm of right common carotid artery	6	2.0	14	None	Bacteriologic cure	2½ yr.	Died suddenly at home in pulmonary edema 2½ years after treatment; no autopsy
4. Ca	37	F	3 mo.	Strep. viridans	0.05	4.0	Rheumatic (MI)	.....	6	2.0	14	None	Bacteriologic cure	4½ yr.	.....
5. Ma	48	M	5 mo.	Microaerophilic streptococcus	0.015	3.1	Congenital (interventricular septal defect)	Bacterial endocarditis 7 years previously	6	2.0	14	None	Bacteriologic cure	20 mo.	Well until 20 months after treatment; died suddenly; autopsy revealed healed endocarditis of septal defect and aortic valve; perforations of all three aortic valve cusps
6. Fa	22	F	2 wk.	Strep. viridans	0.4	3.1	Rheumatic (MI)	.....	6	2.0	16	None	Bacteriologic cure	4½ yr.	.....
7. So	45	M	5 wk.	Strep. viridans	0.1	0.8	Rheumatic (MI, AI)	Probable concurrent active rheumatic fever	6	2.0	14	None	Bacteriologic cure	4½ yr.	Occurrence of petechiae continued more than 2 years after treatment
8. Me	56	M	2 mo.	Strep. viridans	0.1	3.1	Rheumatic (MI)	.....	6	2.0	14	None	Bacteriologic cure	4½ yr.	.....
9. Co	33	F	3–4 mo.	Strep. viridans	0.1	6.2	Rheumatic (MI)	.....	6	2.0	14	None	Bacteriologic cure	4 yr.	.....
10. Far	54	M	9–12 mo.	Strep. viridans	0.2	3.1	Rheumatic (MS, MI, AS, AI)	Bacterial endocarditis 7 years previously; probable bacterial endocarditis 1 year previously	6	2.0	14	None	Bacteriologic cure	2½ yr.	Died suddenly in cardiac failure 2½ years after therapy; no autopsy
11. De	22	F	1 mo.	Strep. viridans	0.2	6.2	Rheumatic (MS, MI, AS)	.....	6	2.0	14	None	Bacteriologic cure	4 yr.	.....

TABLE 1 (*Continued*)

SHORT-TERM PENICILLIN AND DEHYDROSTREPTOMYCIN THERAPY OF STREPTOCOCCAL ENDOCARDITIS. CLINICAL DATA ON 35 PATIENTS

Case No.	Age (yr.)	Sex	Duration of Symptoms before Present Treatment Started	Infecting Micro-organism	Sensitivity		Type of Heart Disease	Associated Conditions	Antimicrobial Therapy (daily dosage)		Days of Treatment	Other Treatment in Follow-up Period	Results of Treatment	Follow-up	Comment
					Penicillin (U/nl.)	Streptomycin (μg./ml.)			Penicillin (million units)	Dihydrostreptomycin (gm.)					
12. Wa	64	F	16 mo.	Strep. viridans	0.1	1.6	Uncertain	.....	1.6	2.0	15	None	Bacteriologic cure	3 yr.	.....
13. Le	52	M	4 wk.	Strep. viridans	0.1	25	Congenital (interventricular septal defect)	Bacterial endocarditis 6 years previously; mild diabetes mellitus	6	2.0	14	None	Bacteriologic cure	4 yr.	.....
14. Sa	20	M	3 mo.	Strep. viridans	0.1	3.1	Rheumatic (MS, MI)	.....	6	2.0	14	None	Bacteriologic cure	3 yr.	.....
15. O'H	40	M	3 wk.	Strep. viridans	.....	....	Rheumatic (MS, MI, AS, AI)	.....	6	2.0	14	None	Bacteriologic cure	3 yr.	.....
16. Wr	56	F	3 mo.	Group H streptococcus	0.05	6.2	Syphilitic (AI)	Neurosyphilis; myocardial infarction in past	6	2.0	14	None	Bacteriologic cure	2½ yr.	Re-treated 1½ years later for possible endocarditis; died 2½ years later with possible endocarditis and cardiac failure; multiple perforations of aortic valve at autopsy
17. Ha	37	F	5 mo.	Strep. viridans	0.4	3.1	Rheumatic (MS, MI)	.....	6	2.0	14	None	Bacteriologic cure	3 yr.	.....
18. Ne	53	F	3 mo.	Strep. viridans	0.05	0.8	Rheumatic (MS, MI)	.....	6	2.0	14	None	Bacteriologic cure	3 yr.	.....
19. Me	58	M	3 mo.	Strep. viridans	0.05	25	Rheumatic (MS)	.....	6	2.0	14	None	Bacteriologic cure	2½ yr.	.....
20. Hau	61	M	6 mo.	Strep. viridans	0.05	6.3	Rheumatic (MS, MI)	.....	6	2.0	14	None	Bacteriologic cure	2 yr.	.....
21. Kra	32	M	3 wk.	Strep. viridans	0.02	6.3	Congenital (interventricular septal defect)	.....	6	2.0	14	None	Bacteriologic cure	3½ yr.	Received 5 days of penicillin therapy prior to admission
22. Fr	48	F	2 mo.	Strep. viridans	0.1	6.3	Rheumatic (MS, MI, AI)	.....	6 for 9 days; 3 for next 5 days	2.0	14	None	Bacteriologic cure	2 yr.	.....
23. Wo	17	F	4 mo.	Strep. viridans	0.05	1.6	Tetralogy of Fallot (Taussig-Blalock operation 8 years previously)	Staphylococcal endocarditis 3 years previously	6	1.0 first week; 2.0 second week	14	None	Bacteriologic cure	1½ yr.	.....

TABLE I (*Continued*)

SHORT-TERM PENICILLIN AND DEHYDROSTREPTOMYCIN THERAPY OF STREPTOCOCCAL ENDOCARDITIS. CLINICAL DATA ON 35 PATIENTS

Case No.	Age (yr.)	Sex	Duration of Symptoms before Present Treatment Started	Infecting Micro-organism	Sensitivity		Type of Heart Disease	Associated Conditions	Antimicrobial Therapy (daily dosage)		Days of Treatment	Other Treatment in Follow-up Period	Results of Treatment	Follow-up	Comment
					Penicillin (U/ml.)	Streptomycin (μg./ml.)			Penicillin (million units)	Dihydrostreptomycin (gm.)					
24. Ka	36	M	3 mo.	Group H streptococcus	0.1	12.5	Rheumatic (MI)	Bacterial endocarditis 5 years previously	6	2.0	14	None	Bacteriologic cure	1 yr.	.....
25. Ve	58	F	3 mo.	Strep. viridans	0.2	4.0	Rheumatic (MI, MS)	Thrombophlebitis	0.6	2.0	10	Penicillin, beginning 1 month after completion of this treatment	Probable bacteriologic cure	3 yr.	Fever 1 month after therapy; received penicillin despite lack of signs of relapse; fever probably caused by thrombophlebitis
26. Gr	48	M	5 mo.	Strep. viridans	0.05	1.6	Undetermined	Myocardial infarction 12 years previously	1.2 for 4 days; 6 for next 14 days	2.0	18	Penicillin beginning 5 weeks after this course of treatment	Probable bacteriologic cure	4 yr.	Apparently well and blood cultures negative in the month after therapy; then bilateral embolic occlusion of retinal arteries; although no bacteriologic evidence of relapse therapy was resumed
27. Fac	57	F	6 wk.	Strep. viridans	.....	....	Rheumatic (AS, AI)	Cirrhosis of liver and organic psychosis; nephritis	0.3 (procaine) for 4 days; 6 for next 14 days	2.0 (last 14 days)	18	Penicillin and dihydrostreptomycin for 14 days beginning 1 month after this treatment	Probable bacteriologic cure	3½ yr.	Slight fever and continuing signs of nephritis subsequent to treatment; although no bacteriologic evidence of relapse was obtained, a second 14-day course of combined therapy was given
28. Bl	41	F	2 wk.	Strep. viridans	0.1	0.8	Rheumatic (MI)	.....	6	2.0	14	Penicillin and dihydrostreptomycin beginning 12 days after this treatment	Probable bacteriologic cure	3 yr.	Twelve days subsequent to treatment severe apical dental abscess developed and antimicrobial therapy was resumed
29. Ver	23	F	6 wk.	Strep. viridans	0.1	0.8	Rheumatic (MS, MI)	Nephritis	6	2.0	14	Oral penicillin beginning 8 weeks after this treatment	Probable bacteriologic cure	2½ yr.	Because of continuing signs of nephritis, oral penicillin given beginning 8 weeks after treatment

TABLE I (*Continued*)

SHORT-TERM PENICILLIN AND DEHYDROSTREPTOMYCIN THERAPY OF STREPTOCOCCAL ENDOCARDITIS CLINICAL DATA ON 35 PATIENTS

Case No.	Age (yr.)	Sex	Duration of Symptoms before Present Treatment Started	Infecting Micro-organism	Sensitivity		Type of Heart Disease	Associated Conditions	Antimicrobial Therapy (daily dosage)		Days of Treatment	Other Treatment in Follow-up Period	Results of Treatment	Follow-up	Comment
					Penicillin (U/nl.)	Streptomycin (μg./ml.)			Penicillin (million units)	Dihydrostreptomycin (gm.)					
30. Au	60	F	4 mo.	Strep. viridans	0.1	3.1	Rheumatic (MI, AI)	.....	6 for 3 days; 3 for next 11 days	2.0	14	Penicillin and dihydrostreptomycin for 5 days, 1 month after this treatment	Probable bacteriologic cure	1 yr.	Cellulitis of face one month after treatment; received 5 days of antimicrobial therapy; no evidence of relapse
31. Ro	64	F	4 mo.	Strep. viridans	0.002	0.01	Rheumatic (MS, AS)	Bleeding carcinoma of colon	4.8	2.0	15	Penicillin and dihydrostreptomycin 3 weeks after this treatment	Probable bacteriologic cure	9 mo.	Urinary tract infection 3 weeks after treatment; received 8 days of antimicrobial therapy; no evidence of relapse; reinfection endocarditis (hemolytic streptococcus) 9 months later
32. Kro	59	F	3 mo.	Strep. viridans	0.2	1.6	Rheumatic (AS, AI)	Congestive heart failure	6	2.0	14	None	Probable bacteriologic cure	3 wk.	Died of cardiac failure 3 weeks after treatment; cultures of heart's blood and of healing vegetations on valves negative at autopsy; perforation of aortic valve cusp
33. Ri	67	M	6 wk.	Strep. viridans	0.1	1.6	Rheumatic (MI)	.....	6	2.0	14	.....	Bacteriologic relapse 15 days posttreatment	.....	Blood culture taken 15 days after completion of treatment positive for Strep. viridans; patient re-treated with penicillin
34. Aq	24	M	3 mo.	Strep. viridans	0.05	0.2	Rheumatic (MI, MS, AI, AS)	Possible mycotic aneurysm of femoral artery	6	2.0	14	.....	Bacteriologic relapse (twice) 14 days and 6 days post-treatment	.....	Relapsed after first course; was re-treated in the same way and again relapsed; subsequently cured with 6 weeks of penicillin therapy
35. Ra	20	M	5 mo.	Strep. viridans	0.013	6.2	Congenital (interventricular septal defect; pulmonic stenosis)	.....	3	2.0	14	.....	Bacteriologic relapse 10 days post-treatment	.....	Re-treated successfully with penicillin alone for 6 weeks

by 0.1 unit/ml. or less. Twenty-nine strains were sensitive to streptomycin in concentrations of 6.2 μg./ml. or less; one strain was sensitive to 8 μg./ml., one to 12.5 and one to 25 μg./ml. Two strains were not tested but were presumed to fall into the penicillin-sensitive category on the basis of general characteristics of the organism and the response of the patients to treatment.

The basic regimen of therapy employed was as follows: the concurrent administration for a period of fourteen days of 500,000 units potassium penicillin G intramuscularly every two hours (total daily dose: 6 million units) and 0.5 gm. dihydrostreptomycin four times a day or 1.0 gm. twice a day intramuscularly (total daily dose: 2 gm.). Minor variations in this regimen which occurred in some cases are indicated in Table I. These were mainly variations in the daily amount of penicillin given, and are not considered to have significantly influenced the results. No patient received a total daily dose of penicillin greater than 6 million units. Following the completion of treatment blood cultures were usually taken three times a week for the ensuing two weeks, and at longer intervals thereafter for at least three months. The patients were observed closely for evidence of relapse throughout the follow-up period. The majority are still being seen at regular intervals.

RESULTS

These results of treatment are summarized in Table I in which the cases are grouped according to outcome rather than by historic sequence.

Of the thirty-five patients treated, bacteriologic cure was achieved beyond reasonable doubt in twenty-four (Cases 1 to 24 inclusive), i.e. the patient's response to therapy was entirely favorable, blood cultures were consistently sterile throughout treatment and for at least three months thereafter, and no additional antibiotic therapy was given for any reason.

In eight cases (Cases 25 to 32 inclusive) the result has been classed as "probable bacteriologic cure." In four of these cases there was persistent fever or other clinical signs of possible continued infection which prompted further antibiotic therapy despite sterile blood cultures. In three patients further antibiotic therapy was necessary during the post-treatment period because a new and unrelated infection. In one instance (Case 32) death occurred only three weeks after the completion of therapy and, although there had been no clinical or laboratory evidence of continued or recurrent infection and none was found at autopsy, the case is classed as a "probable bacteriologic cure" because of the short follow-up period. Thus in twenty-four cases (69 per cent) the result was definite clinical and bacteriologic cure, and in eight (23 per cent) it is classed as probable cure. In thirty-two cases (91 per cent), then, the result can be classed as certain or probable bacteriologic cure.

In the remaining three cases (Cases 33 to 35 inclusive), or 8.6 per cent of the total, bacteriologically proved relapse occurred. One patient (Case 34) relapsed twice following short-term combined therapy. If calculated on the basis of four relapses in thirty-six courses of therapy, therefore, the incidence of relapse is 11.1 per cent. Because of their importance, brief summaries of these three cases are presented.

CASE REPORTS

CASE 33. Ri. This sixty-seven year old man was admitted to the New York Hospital on March 12, 1952, with low back pain of three weeks' duration. There had been unusual fatigue, anorexia and thirst for six weeks. Physical examination revealed a temperature of 38.7°C. and an enlarged heart with signs of mitral stenosis and insufficiency. Viridans streptococci (50 to 60 colonies per ml.) were recovered from three successive blood cultures. This organism was inhibited *in vitro* by penicillin in a concentration of 0.1 unit/ml., and by streptomycin in a concentration of 1.6 μg./ml. From March 18 to April 1 (fourteen days) he was treated with 500,000 units of potassium penicillin G every two hours and 0.5 gm. dihydrostreptomycin every six hours, both intramuscularly. The response was satisfactory but two days following the completion of therapy his temperature rose to 38.5°C. On the following day, at his insistence, he was discharged from the hospital. He returned twelve days later and, although apparently well, blood culture taken at that time (fifteen days post-treatment) again yielded viridans streptococci (10 colonies per ml.). This strain was not significantly changed from the one originally isolated, in respect to *in vitro* sensitivity to penicillin or streptomycin. (Table II.) The patient was re-treated in another country but has been lost to further follow-up examinations here.

CASE 34. Aq. This twenty-four year old man with known rheumatic heart disease was admitted to the New York Hospital in July 1953 because of bouts of fever, tender erythematous swellings on the extremities, and weight loss for three months. The heart was enlarged, with signs of stenosis and insufficiency of both the mitral and aortic valves. Viridans strepto-

TABLE II
ORGANISM "SENSITIVITY" IN VITRO

Strain	Case 33		Case 34		Case 35	
	Penicillin (unit/ml.)	Streptomycin (μg./ml.)	Penicillin (unit/ml.)	Streptomycin (μg./ml.)	Penicillin (unit/ml.)	Streptomycin (μg./ml.)
Original strain	0.1	1.6	0.05	0.2	0.013	6.2
First relapse strain	0.1	0.8	0.1	0.2	0.025	3.1
Second relapse strain	...	...	0.05	0.8	.....	...

cocci were recovered from three successive blood cultures. This organism was inhibited *in vitro* by penicillin in a concentration of 0.05 unit/ml. and by streptomycin in a concentration of 0.2 μg./ml. From July 22 to August 5, 1953 (fourteen days) the patient was treated with 6 million units of potassium penicillin G during each twenty-four-hour period by continuous intravenous drip and 1.0 gm. of dihydrostreptomycin every twelve hours administered intramuscularly. Although his course was satisfactory during treatment, the temperature rose gradually to 38.4°C. at the end of the second week of post-treatment, and blood cultures taken at that time yielded viridans streptococci (25 to 35 colonies per ml.). For the first time a thrill was noted over the right femoral trigone. No mass developed here, however, and the signs remained unchanged throughout the remainder of his hospital stay. Although it was thought possible that a mycotic aneurysm of the femoral artery had developed, the physical signs of this were never conclusive.

A second course of combined therapy was administered, identical with the first except that the penicillin was given intermittently every two hours rather than continuously. Six days following the completion of the second course of therapy, however, several blood cultures again were positive for the streptococcus (5 to 6 colonies per ml.). A third course of treatment consisting of 800,000 units of procaine penicillin administered intramuscularly twice daily for forty-two days resulted in clinical and bacteriologic cure. The streptococcal strains isolated from the blood stream following the two unsuccessful courses of combined therapy exhibited no significant change in susceptibility to penicillin or streptomycin *in vitro*. (Table II.)

CASE 35. Ra. This twenty year old man was admitted to the New York Hospital on April 11, 1954, with exertional dyspnea, malaise and chills of five months' duration. He was known to have a congenitally malformed heart with an interventricular septal defect and pulmonic stenosis. The temperature was 39.3°C., petechiae were present in the conjunctiva and splinter hemorrhages in the nail beds. A viridans streptococcus (125 to 150 colonies per ml.) was recovered from three successive blood cultures. This organism was inhibited *in vitro* by penicillin in a concentration of 0.013 unit/ml. and by streptomycin in a concentration of 6.2 μg./ml. From April 15 to April 29, 1954 (fourteen days) he received 250,000 units of potassium penicillin G every two hours and 1.0 gm. of dihydrostreptomycin every twelve hours, both administered intramuscularly. One week following the conclusion of therapy a soft diastolic murmur was first noted over the base of the heart and, although there was no other evidence of continued active infection, a viridans streptococcus was recovered from one of six blood cultures taken at that time. The patient was successfully re-treated with penicillin alone intramuscularly for six weeks. During re-treatment the aortic insufficiently became severe, suggesting aortic valve cusp perforation. Subsequently a Hufnagel prosthesis was inserted into the thoracic aorta, with some functional improvement. The streptococcus which was recovered following the unsuccessful course of combined therapy showed no significant change in sensitivity to penicillin or streptomycin when compared with the original strain. (Table II.)

COMMENTS

The results presented here afford additional clinical evidence for the decided effectiveness of the combination of penicillin and dihydrostreptomycin in the treatment of bacterial endocarditis caused by viridans streptococci and other streptococci which are reasonably sensitive to penicillin *in vitro*. The frequency of bacteriologic cure with the first course of treatment in this series (91 per cent) is quite comparable to that generally observed with the use of penicillin alone for twenty-eight days, and very probably is considerably higher than would be encountered with penicillin alone given in this dosage for only fourteen days (see footnote page 58).

However, the four instances of proved bacteriologic relapse which occurred in three patients in this series (to our knowledge the first reported instances of bacteriologic relapse

following such therapy) indicate that this therapy is not curative in all cases, and that even with the use of this effective drug combination there is need for prolonged therapy in some patients with this disease.

The results serve to focus our attention again on the nature of the lesion and on the host-parasite-antibiotic dynamics in this rather unique infectious disease. Several aspects have received study by others. That penicillin kills rapidly multiplying bacteria much more rapidly than slowly multiplying or resting bacteria was discovered by Hobby and her associates [*18,19*]. The importance of the density of the bacterial population and the metabolic and nutritional state of its members in influencing the bactericidal effect of penicillin has been further investigated by Eagle and others [*20–23*]. Eagle [*21*] states: "Indeed, whenever an inflammatory focus supplies suboptimal conditions for growth, one may anticipate a slower bactericidal action than is observed under optimal conditions *in vitro;* and it may require a longer time period to effect cure with penicillin than would be necessary to kill the corresponding number of organisms in the test tube." These considerations would appear to be applicable to the lesion of subacute bacterial endocarditis, and it seems probable that Eagle's statement applies to the effect of a combination of penicillin and streptomycin as well as of penicillin alone on viridans streptococci, as indeed is suggested by some experiments of Hunter [*12*].

The importance of the penicillin level in the tissues and of the interval between doses in obtaining maximal killing effect and in influencing the necessary duration of treatment has been studied for group A, B, C and D streptococci, viridans streptococci and certain other organisms by Eagle [*21,22*]. It seems possible that the penicillin zone phenomenon is of some clinical importance in the treatment of streptococcal endocarditis [*9,24*].

We have already commented upon the many studies, both laboratory and clinical, which have presented evidence for the greater bactericidal effect of penicillin and streptomycin on streptococci and have thrown some light on the mechanism of this phenomenon.

The possible role of the outer fibrin layer of the vegetation in the prevention of diffusion of antibiotics into the infected area has received study. It does not appear to be a significant factor with penicillin or streptomycin [*25,26*]. However, this fibrin layer and the central necrotic layer, between which the bacteria lie, are both relatively impervious to leukocytes [*27*]. According to Moore [*27*] leukocytes do not reach the bacteria in significant numbers until the superficial fibrin layer of the vegetation has been replaced to some extent by capillaries and fibroblastic tissue. Those viridans streptococci which are phagocytized are probably killed, since it has been demonstrated experimentally, for several strains at least, that these bacteria do not survive phagocytosis as do pathogenic staphylococci [*28*].

Some strains of viridans streptococci and group H streptococci produce high polymer dextrans and levans *in vitro* [*29*]. These substances may conceivably also be produced *in vivo* and may play a role in the genesis of the lesion and in the modification of the host's response. In this connection the results of Shilo et al. [*30*] and Davies et al. [*31*] suggesting that these substances may hinder the passage of leukocytes and antibodies through vessel walls are of considerable interest.

Homologous agglutinating, precipitating and complement-fixing antibodies are known to be present in the serums of many patients with subacute bacterial endocarditis and of animals experimentally infected with non-hemolytic streptococci [*32–34*]. However, even though they may be present in high titer they appear to be quite ineffective in bringing about recovery from the infection. The interesting possibility that immune processes may even play a role in the genesis of bacterial endocarditis [*35,36*] remains to be clarified.

Lastly, it seems reasonable to suppose that the size and extent of the lesions in a given patient may be of importance in determining the length of time required for curative treatment. Assuming the type of infecting organism to be constant, a patient with only one or two small vegetations on one valve should be easier to cure than one with multiple large vegetations involving valves, chorda tendinae and mural endocardium. Although this may well be true, it is not possible to estimate accurately the extent of disease during life. The duration of the illness does not seem to be a reliable index of it, at least insofar as it may be reflected by the response to chemotherapy. In this series, for instance, the average duration of illness for all patients prior to therapy was thirteen weeks; the duration of the illness in the three bacteriologic failures was

six, twelve and twenty weeks, an average of thirteen weeks. The apparent lack of correlation between the duration of illness and the ease of bacteriologic cure has been commented on by others [*10*].

The results reported in this paper indicate that, even if the most bactericidal drug combination now available for this group of organisms is administered, prolonged therapy is necessary to achieve final eradication of the infection in some patients. As it is not possible at this time to predict in which patients short-term treatment will fail, it is suggested that it may be advisable to extend treatment for an additional seven to fourteen days beyond the initial two weeks, employing either penicillin alone or further combined therapy. By this regimen it may prove possible to cure the remaining 10 per cent of patients, and the risk of dihydrostreptomycin toxicity should not be significantly increased.

Other less persuasive but nonetheless practical arguments for extension of the routine treatment of subacute bacterial endocarditis beyond fourteen days are: (1) the long time which is necessary for complete healing to occur, apparently a period of several months [*27*], (2) the significant incidence of associated myocarditis [*37–40*], and (3) the practical matter of the management of the patient with clinical signs possibly indicative of continued active infection during the third and fourth weeks, a source of some doubt and confusion in several cases in this series. If treatment was begun with the intention of continuing it for four weeks this period of doubt as to whether or not the patient has been cured would be largely avoided. These are admittedly less compelling reasons for the extension of treatment than the observation of a significant number of relapses, and the latter finding is the real basis for our suggestion that treatment be extended beyond two weeks.

It is of interest that the strains isolated following unsuccessful short-term combined therapy exhibited no significant increase in resistance to either penicillin or streptomycin *in vitro*. In the case of penicillin no increase in resistance was to be expected, but the emergence of strains highly resistant to streptomycin is the rule following unsuccessful therapy with the latter drug [*1,7*]. This emergence was apparently prevented in the cases reported here by the use of combined therapy, an observation which affords clinical confirmation of the experimental results of Wright et al. [*41*].

SUMMARY

Results are presented of the treatment of thirty-five patients with penicillin-susceptible streptococcal endocarditis by a fourteen-day course of concurrent penicillin and dihydrostreptomycin. Definite bacteriologic cure was achieved in twenty-four (69 per cent) and probable bacteriologic cure in an additional eight (23 per cent), a total of thirty-two (91 per cent) definitely or probably cured. Bacteriologic relapse was observed in three patients (approximately 10 per cent), one of whom relapsed twice.

It is concluded that concurrent penicillin and dihydrostreptomycin probably represents the most bactericidal antimicrobial therapy available today for this group of infections. The use of this therapy, however, for a period as short as fourteen days falls short of the goal of antimicrobial therapy, which is bacteriologic cure in *all* cases. It is suggested, accordingly, that as it is impossible to predict which patients will not achieve cure with short-term therapy, it may be advisable to extend the routine therapy of this disease an additional seven to fourteen days in the hope that the remaining 10 per cent of patients will be cured by the first course of therapy. Some possible factors in the need for prolonged therapy in some patients with bacterial endocarditis are cited.

There was no significant change in the *in vitro* sensitivity to either penicillin or streptomycin in the strains isolated following unsuccessful short-term combined therapy, indicating that concurrent administration of the two antibiotics prevented the emergence of streptomycin resistance.

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ACTH and Adrenal Steroids in the Treatment of Pneumococcal Meningitis in Adults*

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THE outlook in pneumococcal meningitis has steadily improved since the introduction of sulfonamides [*1*]. The best results have been obtained by Nemir and Israel [*2*], who reported 93 per cent recovery in a series of children with pneumococcal meningitis treated with a combination of penicillin and sulfonamides, but almost equal success has been achieved with similar therapy by others in patients under the age of twenty [*3,4*]. Results of treatment of pneumococcal meningitis in older persons have been less satisfactory, however. Feibush [*5*], for example, treated twenty-two adults with a combination of large doses of penicillin and sulfonamides and only 32 per cent recovered. Similar low rates of survival are apparent in other reports of therapy in adults with pneumococcal meningitis. (Table I.)

This failure of therapy in a large proportion of adults has continued despite the use of antibacterial drugs which are known to sterilize the spinal fluid and is believed, therefore, to arise from the presence of injurious factors other than the living pneumococcus. Because pressure from the inflammatory exudate might be lethal, and because clinical [*16*] and experimental [*17*] work indicate that ACTH and cortisone suppress the inflammatory reaction to pneumococci, a group of twelve consecutive cases of pneumococcal meningitis in adults were treated with these drugs in combination with penicillin in an attempt to increase the percentage of cures. The results described in this report indicate that such combined use of ACTH or adrenal steroids and penicillin is superior to the use of penicillin alone. Except for two isolated case reports, the literature contains no other reference to combined hormone-antibiotic therapy in pneumococcal meningitis [*18,19*].

DESCRIPTION OF PATIENTS

The treatment of each patient in this report was supervised by us. All patients were adults with pneumococcal meningitis, hospitalized at Parkland Memorial Hospital between January, 1954, and February, 1957, except for one patient at the Veterans Administration Hospital in McKinney, Texas. The pneumococcus was demonstrated in the spinal fluid of all patients by direct examination and isolated in culture from eleven of them. Blood cultures were also performed in ten patients and the pneumococcus isolated from nine. The sterile blood culture was obtained from the man who had received antibiotics before admission.

A source of infection was found in all but three cases; either otitis, sinusitis or a skull defect was present in seven cases and pneumonia in two.

The ages ranged from twenty-seven to seventy-three years. Nine patients were in deep coma and appeared desperately ill. Three others who were semi-comatose could be aroused by strong stimuli and were then able to talk in a disoriented manner. One patient who had received antibiotics before admission was able to sit up, but was disoriented and restless. Four patients suffered peripheral vascular collapse early in their hospital course, and two required neurosurgical procedures for decompression.

The infections described in Case X and in Case XII occurred in the same patient and were both due to type 17 pneumococcus. The second bout of meningitis occurred four months after the first and was thought to represent reinfection from carrier sources in the patient's family rather than from persistence of the organism in the spinal fluid. Failure of type-specific immunity to result from pneumococcal infection

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TABLE 1

SUMMARY OF REPORTED RESULTS OF THE TREATMENT OF PNEUMOCOCCAL MENINGITIS WITH PENICILLIN ALONE OR IN COMBINATION WITH SULFONAMIDES

Author	No. of Patients	Per cent Recovery			Treatment
		Total	Under Age 20	Over Age 20	
Smith et al. [6], 1946	19	63	*	*	Penicillin, intramuscularly and intrathecally
	19	89	*	*	Sulfonamides; penicillin, intramuscularly and intrathecally
Ross and Burke [3], 1946	19	84	84	†	Sulfonamides, penicillin, intramuscularly and intrathecally
Waring and Weinstein [7], 1946	9	11 (0/2)	0	14 (1/7)‡	Penicillin, intramuscularly and intrathecally
	18	83	100 (4/4)	79 (11/14)	Sulfonamides; penicillin, intramuscularly and intrathecally
Lowrey and Quilligan [8], 1948	5	80	*	*	Sulfonamides; penicillin, intramuscularly and intrathecally
	17	82	61 (10/13)	100 (4/4)	Sulfonamides; penicillin, intramuscularly and intrathecally
Ruegsegger [9], 1949	158	51	*	*	Penicillin
	224	71	*	*	Penicillin, sulfonamides
Meneely [10], 1949	25	72	88 (8/9)	62 (10/16)	Sulfonamides; penicillin, intramuscularly and intrathecally
Dowling et al. [11], 1949	21	62	40 (2/5)	68 (11/16)	Penicillin 1,000,000 units every 2 hours, intramuscularly; 13 received sulfonamides; 3 patients received penicillin, intrathecally
Applebaum et al. [4], 1949	125	74	86 (44/51)	65 (48/74)	Penicillin, intramuscularly and intrathecally; sulfonamides
Jackson [12], 1950	163	28	*	*	Penicillin, intramuscularly and intrathecally; sulfonamides; only 24 patients were considered to have had adequate therapy
Simpson and Holley [13], 1951	40	60	60 (9/15)	60 (15/25)	Penicillin, intramuscularly and intrathecally; sulfonamides
Nemir and Israel [2], 1951	15	93	93	†	Penicillin, intramuscularly and intrathecally; sulfonamides
Lepper and Dowling [14], 1951	43	70	72 (8/11)	68 (22/32)	Penicillin, intramuscularly only, 12,000,000 units/day
Feibush et al. [5], 1952	22	32	100 (2/2)	25 (5/20)	Penicillin up to 12,000,000 units/day; penicillin intrathecally to some, including those who died
Bunn and Peabody [15], 1952	20	75	75 (6/8)	75 (9/12)	Penicillin, intramuscularly, at least 12,000,000 units/day in adults; no sulfonamides

* Number under and over 20 years not apparent from the data given.

† No adults.

‡ Numerator—total number of survivors; denominator—total number of patients.

has been described by Finland and Winkler [*20*] and is best explained by the concept that the incubation period of the disease is too short to permit the development of an anamnestic immune reaction [*21*].

TREATMENT

The usual dose of penicillin was 20 to 30 million units daily given in the form of a slow intravenous drip. In three patients, however, part of the daily total was given intramuscularly in divided doses of 1 or 2 million units every two hours. All patients received ACTH and three received hydrocortisone intravenously in the doses stated in the case reports; one received cortisone intramuscularly. The ACTH or hydrocortisone were added to the same bottle of intravenous fluids containing the penicillin. Individual variations in administration of antibiotics, ACTH and adrenal steroids are described in conjunction with other therapeutic measures in the detailed case histories which follow.

ACTH was used in all patients to avoid the possibility of adrenal atrophy which may follow the use of cortisone and other adrenal steroids. Numerous reports on the effectiveness of ACTH in bringing about clinical improvement in severe infections have established that the adrenal cortex can be stimulated to activity beyond that resulting from the infection itself [*16,22–25*].

CASE REPORTS

Case I (54720). This twenty-seven year old man was admitted to this hospital for the second time in 1956. In 1946 he was treated with penicillin for meningitis occurring after skull fracture. In 1948 he was admitted to the hospital for the first time for treatment of meningitis. The smear of the spinal fluid then showed gram-positive diplococci, but cultures were sterile. He was treated with penicillin and sulfonamides and made a satisfactory recovery.

The patient was admitted for the second time in November, 1956, after transfer from another hospital where he had received unknown quantities of tetracycline, streptomycin and penicillin. On admission he was irritable and thrashing about.

Physical examination revealed the temperature to be 100°F.; pulse, 94; blood pressure, 140/95 mm. Hg. The neck was stiff, the pupils reacted to light. The examination of the heart and lungs was normal. The deep tendon reflexes were equal and active and the superficial reflexes were present.

An x-ray film of the chest showed no pneumonia or other abnormalities. The white blood cells numbered 54,100 per cu. mm. with 82 per cent segmented neutrophils, 5 per cent non-segmented neutrophils, 12 per cent lymphocytes, and 2 per cent monocytes. The urinalysis showed 1 plus albumin, traces of sugar and acetone. The spinal fluid contained moderate numbers of gram-positive, encapsulated, lancet-shaped diplococci; these were identical in morphology to pneumococci but did not type with antipneumococcal serum.

Table II—Treatment

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)	Hydrocortisone, Intravenously (mg.)
0	30	40	200
1	20	40	100
2	30	60	0
3	30	60	0
4	30	0	0
5	30	0	0

After eight hours of treatment the temperature was 99°F., and within sixteen hours the patient was mentally clear. On day 1 he was taking a diet and felt well. He was discharged on the ninth hospital day. (Table II.)

Case II (11951). This thirty-four year old Negro woman was brought to the hospital in a coma. One week before her admission a "cold" developed followed by chills and fever. The day before she was brought to the hospital she gradually became disoriented and unconscious.

Physical examination revealed the temperature to be 102.2°F; pulse, 100; respiration, 32. Her neck was stiff. On examination of the chest there was dullness to percussion over the right upper lobe of the lung, increased breath sounds and rales. The reflexes were normal.

An x-ray of the chest showed "bronchopneumonia involving the right upper lobe."

On admission the hemoglobin was 6.3 gm./100 ml., the white cell count was 20,000 per cu. mm. with 6 per cent lymphocytes, 81 per cent segmented neutrophils and 13 per cent non-segmented neutrophils. Type 6 pneumococcus was demonstrated in the spinal fluid by capsular swelling with specific antiserum.

Therapy during the first twenty-four hours consisted of 60,000,000 units of regular penicillin, 40 units of ACTH, and 150 mg. of cortisone intravenously. The treatment is summarized in Table III.

The penicillin therapy was continued for a period of three weeks.

Because of low urine output, the administration of potassium salts was not begun until twenty-four hours after admission. At this time the serum potassium

concentration was 2.9 mEq./L., the pH of the blood 7.52 and it was learned that she had had diarrhea before entering the hospital. Administration of parenteral potassium chloride corrected this disturbance.

TABLE III—TREATMENT

Day	Penicillin, Intravenously, (millions of units)	ACTH, Intravenously (units)	Hydrocortisone, Intravenously (mg.)
0	60	40	150
1	30	40	0
2	30	40	0
3	15	0	0
4	35	0	0
5	20	0	0
6	30	0	0
7	20	0	0

Eight hours after admission she had two generalized convulsions which were controlled by barbiturates. Irregular rapid respirations were corrected during the first day by lowering the spinal fluid pressure with repeated taps, and upper airway obstruction was treated with tracheostomy. Persistent tachypnea and episodes resembling carpo-pedal spasm were also present. For the first two weeks the course was complicated by a negative sodium balance, attributed to cerebral salt wasting.

Improvement was gradual but marked. By the third hospital day the patient was able to follow simple commands; by the fifth day she was more alert and talked feebly; and by the seventh hospital day the patient was able to drink. Upon discharge, thirty days after admission, the patient was well oriented, alert and cooperative. Her only complaint was moderate weakness.

She was seen several months later in the outpatient department with no residual lesions.

CASE III (66403). A thirty-seven year old Negro woman entered the hospital in a stuporous condition and was unable to give a history. Four days before admission she had complained to her family of bilateral frontal headaches, feverishness and chilly sensations. The day before admission she complained of a stiff neck and pain behind her right ear.

Physical examination revealed the temperature to be 99°F.; pulse, 112; respiration, 18; and blood pressure, 140/90 mm. Hg.

The patient was stuporous, irrational and exhibited marked nuchal rigidity. The right auditory canal was constricted and examination of the right tympanum was impossible. There were no abnormal findings in the lungs or heart, and reflexes were normal.

Type 25 pneumococcus was demonstrated in the spinal fluid by capsular swelling. The same type of pneumococcus was cultured from the blood.

X-ray showed bilateral clouding of the mastoids, but no pneumonia.

TABLE IV—TREATMENT

Day	Penicillin, Intravenously (millions of units)	Penicillin, Intramuscularly (millions of units)	ACTH Intravenously (units)	ACTH Intramuscularly (units)
0	10	8	25	200
1	10	24	0	200
2	0	24	0	200
3	0	24	0	200
4	0	24	0	200
5	0	24	0	200
6	0	24	0	200
7	0	24	0	200

The dose of ACTH was reduced gradually until the seventeenth hospital day when it was discontinued. Small doses of penicillin were given for three weeks. (Table IV.)

For the first three days in the hospital the patient was restless and almost maniacal. By the fourth day she was rational, ate without assistance and walked to the bathroom. She remained symptom-free thereafter.

CASE IV (75590). This forty-four year old man with an old skull fracture was admitted to Parkland Memorial Hospital for the third time complaining of chills and fever for six hours. In 1941 he was admitted to another hospital with a skull fracture of the "left side of the skull with extension into the left frontal sinus" and a fascia lata transplant was made to cover the defect in the dura. The patient returned home in good condition, but clear watery fluid often ran from his nose.

In 1950 he was admitted to an osteopathic hospital because of chills, fever and throbbing headache. Four days after antibiotic therapy was begun, a spinal tap was performed which was said to show "meningococci." He was then transferred to Parkland Memorial Hospital where the spinal fluid contained a low concentration of sugar, 300 cells, but no bacteria. He was treated with penicillin and sulfonamides. By the eighth day the cells had disappeared from the spinal fluid and the patient appeared to be well. (Table V.)

Physical examination revealed the temperature to be 105.6°F.; pulse, 126; respirations, 60; blood pressure, 180/104 mm. Hg.

The patient was semi-comatose, moved about in a purposeless manner and was extremely disoriented. The neck was stiff; the pupils were small and regular and reacted to light. The examination of the fundus was not unusual. No abnormalities were revealed in

the examination of the chest. The neurologic examination could not be performed at the time of admission.

The white blood cell count on admission was 19,400 with 13 per cent lymphocytes, 65 per cent segmented neutrophils, 13 per cent non-segmented neutrophils. The urine contained 2 plus albumin, 1 plus sugar and a few red cells. Type 33 pneumococci were present in the spinal fluid.

TABLE V—TREATMENT

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)
0	45	0
1	45	120
2	30	80
3	30	80
4	30	80
5	30	80
6	30	0
7	30	0
8	30	0
9	30	0

By day 1 the fever had disappeared but the man was still unresponsive. By day 2 the patient responded somewhat to spoken commands and by day 4 he ate his meals. Shortly afterwards herpetic vesicles appeared on his lips.

The patient returned to work and remained well until seven months after his discharge from the hospital when he experienced a Jacksonian convulsion.

CASE V (109226). This forty-six year old alcoholic white woman was in deep coma when she entered the hospital. A neighbor said the patient had been ill for a week and had vomited several times.

On physical examination the temperature was 104.6°F.; pulse, 120; respirations, 48; blood pressure, 140/90 mm. Hg.

She was totally unresponsive to painful stimuli. The neck was rigid and the dilated pupils did not contract when exposed to light.

Hyperresonance and coarse rhonchi were present over both lungs. In the right lung base and in the right axilla there were many coarse rales. Except for tachycardia the heart was normal.

The neurologic examination showed a vertical nystagmus, equal and hyperactive deep tendon reflexes and no Babinski reflexes.

An x-ray of the chest taken on admission was interpreted as "consolidation of a portion of the right upper lobe with cavitation." An x-ray film of the skull was thought to be normal.

On admission the hemoglobin was 10 gm./100 ml. The white blood count was 23,600 with 8 per cent lymphocytes, 73 per cent segmented neutrophils and 19 per cent non-segmented neutrophils. A marked acetonuria and slight glucosuria were present.

In the spinal fluid many gram-positive diplococci were seen and capsular swelling was produced with type 14 anti-pneumococcal serum. Type 14 pneumococcus was also cultured from the blood.

Initial therapy is outlined in Table VI.

TABLE VI—TREATMENT

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)
0	20	80
1	30	40
2	20	40
3	20	80
4	20	0
5	20	0
6	30	0
7	20	0

The patient received 20 to 30 million units of penicillin intravenously daily for eighteen days and 8 million units a day intramuscularly for the treatment of an abscess of the lung.

Twelve hours after treatment with 20 million units of penicillin and 80 units of ACTH, the patient began to have convulsions on the right side and her blood pressure fell to 80/40 mm. Hg. A tracheostomy was performed for relief of upper airway obstruction, and the patient was maintained on intermittent positive pressure breathing. Continuous infusion of l-norepinephrine was begun.

Because of increased spinal fluid pressure and unilateral convulsions it was decided to perform an operation for decompression. Bilateral craniotomy was carried out and polyethylene catheters were left in the ventricles.

The blood pressure was maintained with l-norepinephrine until day 2 when it was possible to discontinue it. The patient was deeply comatose until day 5 when she became responsive to painful stimuli. On the sixth day of admission she could answer questions. By day 9 she was able to take liquids by mouth and by day 12 she walked about the ward.

CASE VI (McK VAH 64-126). This fifty year old white man was admitted to the hospital because of headaches and fever. He was well until two days before admission when he had a chill. The next day he noticed aching in his muscles, bones and joints and complained of a severe headache. A physician found him disoriented and referred him to the hospital. He was a confirmed alcoholic.

On physical examination the temperature was 104.6°F.; blood pressure, 180/110 mm. Hg. He was delirious and maniacal. The neck was stiff, the pupils

fixed with no reaction, but the fundus was normal. The ears, heart and lungs were normal. The deep tendon reflexes were hyperactive and Babinski signs were questionably present. There was a coarse tremor of both hands. The hemoglobin was 16.0 gm./100 ml. The white blood count was 17,850 with 1 per cent lymphocytes, 2 per cent monocytes, 71 per cent segmented neutrophils, and 29 per cent non-segmented neutrophils.

No pneumonia was found on roentgen examination of the chest. Therapy is outlined in Table VII.

TABLE VII—TREATMENT

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)
0	50	160
1	30	160
2	20	40
3	20	40
4	20	40
5	20	40

After four days of hallucinations and tremors indicative of delirium tremens, the patient became cooperative and on the fifth day he fed himself. Two months after discharge he complained of dizziness but otherwise appeared to have recovered fully.

CASE VII (70064). This fifty-five year old woman was admitted to the hospital in coma. The first admission was in 1949 for a radical excision of an undifferentiated sarcoma of the left nasal region with removal of the eye and bones of the left orbit. Most of nine subsequent admissions were for plastic procedures to repair the defect.

In 1953 she was admitted with a diagnosis of pneumococcal meningitis and treated successfully with sulfonamides and penicillin.

Physical examination revealed the temperature to be 104°F.; pulse, 120; respirations, 40; blood pressure, 130/80 mm. Hg. She was comatose and unresponsive. There was marked nuchal rigidity. The right pupil was dilated and reacted to light, but the fundus was not visualized because of a cataract. There was pus in the left orbit and in the nose, and bilateral erythema of the tympanic membranes. Bronchial breathing was heard throughout both lungs, but an x-ray of the chest showed no evidence of pneumonia.

The white blood count on admission was 13,000 with 23 per cent lymphocytes, 26 per cent segmented neutrophils and 43 per cent non-segmented neutrophils. The urine contained a trace of sugar and a trace of acetone.

Gram-positive diplococci typical of D. pneumoniae were seen in the spinal fluid and pneumococci were isolated from the spinal fluid on culture, but these did not exhibit capsular swelling with any of the anti-pneumococcal serums.

TABLE VIII—TREATMENT

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)	ACTH, Intramuscularly (units)	Chloramphenicol (gm.)
0	20	0	0	1.0
1	15	80	0	1.0
2	20	20	0	0
3	25	80	60	0
4	20	0	80	0
5	25	0	80	0
6	25	0	80	0
7	20	0	80	0
8	20	0	80	0
9	10	0	40	0
10	10	0	0	0

Penicillin was continued without ACTH until the thirteenth day. (Table VIII.)

Within eight hours after admission the patient's blood pressure fell to 60/0 mm. Hg and there was slight cyanosis. L-nor-epinephrine was administered through a polyethylene catheter inserted into a leg vein, and a tracheostomy was performed. At this time the patient started having generalized convulsions, localized in a few hours to the right side. Burr holes were made, and a polyethylene catheter inserted into the left ventricle for continuous drainage. At the time of the procedure it was noted that the dura was thick and fibrous, presumably as a result of her previous meningitis.

By day 2 the patient was slightly responsive and able to follow movements with her eye. By noon of day 2 the nor-epinephrine was discontinued. The blood pressure was stable at 100/80 mm. Hg.

On days 4 and 5 the patient convulsed without much change in her responsiveness and the catheter was removed from the ventricle.

On day 6 the patient was markedly improved, responded to commands and moved all her extremities; and by day 9 she was able to talk and to sit up to eat.

CASE VIII (111561). This fifty-six year old white man was brought to the hospital with a history of having fainted. He was deeply comatose and no further history was obtained.

On physical examination the temperature was 105°F.; pulse, 130; respirations, 30; blood pressure, 212/110 mm. Hg. The patient was comatose and appeared moribund. The pupils were fixed and nonreactive and the disc margins were blurred bilaterally.

The heart and lungs were normal. The reflexes were all normal.

The hemoglobin on admission was 15.3, the white blood cell count was 17,000 with 7 per cent lymphocytes, 57 per cent segmented forms and 36 per cent non-segmented forms. The urine contained a few red cells, 3 plus sugar, and 1 plus albumin.

Type 7 pneumococcus was identified in the spinal fluid by capsular swelling.

An x-ray of the chest showed slight cardiomegaly but no pneumonia, and an x-ray of the skull was reported as showing an expanding lesion of the brain depressing the floor of the sella turcica.

In addition to antibiotics and steroid drugs, for the first seven days the patient required constant injections of l-nor-epinephrine to prevent severe hypotension and a tracheostomy to maintain a clear airway. (Table IX.)

The patient received 6 to 12 million units of penicillin intramuscularly for the next twenty days. Cortisone was slowly reduced in dosage until the thirteenth hospital day when it was discontinued.

This man continued to spike fever for the remainder of his course in the hospital. For three weeks he remained semi-comatose and was fed by tube.

He was transferred to another hospital where he died six months later of bronchopneumonia. An autopsy disclosed a chromophobe adenoma of the pituitary extending to the meninges, sella turcica and cerebral hemisphere, but no residual lesions of meningitis were found.

TABLE IX—TREATMENT

Day	Penicillin, Intravenously (millions of units)	Penicillin, Intramuscularly (millions of units)	ACTH, Intravenously (units)	Cortisone (mg.)	Streptomycin (gm.)
0	10	12	0	150	0
1	15	12	0	0	2.0
2	15	12	30	100	2.0
3	0	12	0	100	2.0
4	20	12	10	150	2.0
5	5	12	5	150	2.0
6	5	12	5	50	2.0
7	3	12	3	100	2.0

CASE IX (136203). This sixty-four year old Negro woman was brought to the hospital because of lethargy and incoordination for one day. Sixteen hours before admission a headache developed and she became progressively lethargic. Three years previously the patient noted "pus draining from her nose" and was told that she had sinusitis.

Physical examination revealed the temperature to be 103.4°F.; pulse, 84; respirations, 24; blood pressure, 150/95 mm. Hg. She was comatose, completely unresponsive and exhibited periodic generalized tonic and clonic convulsions. The pupils were small and reacted sluggishly. No papilledema was present. The neck was stiff. Rales were present in the base of the lungs but disappeared when secretions were aspirated from the trachea. The deep tendon reflexes were hyperactive and Babinski signs were present bilaterally.

On roentgen examination of the chest generalized cardiomegaly and a possible infiltrate in the upper lobe of the right lung were seen. A film of the sinuses showed an increase in density at the right maxillary antrum.

The hemoglobin was 14.9 gm./100 ml. and the white blood cell count was 13,100 with 15 per cent lymphocytes, 76 per cent segmented neutrophils and 9 per cent non-segmented neutrophils. The urine had a trace of albumin, 3 plus sugar and a strongly positive reaction for acetone. The urea nitrogen was 8 mg./100 ml. and the blood sugar on admission was 245 mg./100 ml.

Three blood cultures drawn at the time of admission yielded innumerable colonies of type 12 pneumococci in the poured plate. Type 12 pneumococci were also identified directly in the spinal fluid by direct capsular swelling.

TABLE X—TREATMENT

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)	Potassium Chloride (mEq.)
0	30	40	40
1	30	20	40
2	30	40	20
3	30	40	20
4	45	40	20
5	30	40	20
6	30	40	0
7	0	30	40

Immediately after admission convulsions were so frequent that she was given phenobarbital and dilantin.® By the next morning (day 1) the convulsions were controlled without anticonvulsants, but the patient was still comatose. Fever subsided within eight hours. Therapy is outlined in Table X.

By day 2 the patient responded to painful stimuli but did not talk. By day 4 the patient was well oriented and could eat and talk. At this time herpetic lesions were noted on the lips. Scrapings of these demonstrated the giant epithelial cells of herpes simplex and the virus was isolated by corneal inoculation of a rabbit.

Case x (146645). This seventy-two year old Negro man was transferred from another hospital where he was taken because of stupor and inability to talk. At the other hospital nuchal rigidity was noted. Many leukocytes were found in the spinal fluid.

On physical examination the temperature was 101.2°F.; pulse, 70; respirations, 16; blood pressure, 100/80 mm. Hg.

On admission he was semi-stuporous, and although responsive to questioning, his answers were irrational. There was slight nuchal rigidity, slight cardiomegaly and the lungs were clear to examination. The cranial nerve functions were intact and all reflexes were normal.

An x-ray of the chest showed scoliosis of the dorsal spine to the right and coarse markings throughout the lung fields. An x-ray film of the skull was normal.

The white blood cell count was 22,400 with 11 per cent lymphocytes, 5 per cent monocytes, 80 per cent segmented neutrophils and 4 per cent non-segmented neutrophils. The urine was not unusual except for traces of albumin and sugar. Type 17 pneumococci were identified directly in the spinal fluid by capsular swelling.

Table xi—Treatment

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)	Hydrocortisone, Intravenously (mg.)
0	40	0	150
1	30	0	150
2	30	40	50
3	30	60	0
4	30	60	0
5	30	60	0
6	30	30	0

This man demonstrated perhaps the most striking response to therapy of all patients in this series. Eight hours after admission he was afebrile, completely rational and was able to sit up to eat his meals.

Fever did not recur; he was discharged on the ninth hospital day. (Table xi.)

Case xi (99636). This seventy-three year old white man was found unconscious. No other history was available when he arrived at the hospital.

He had been treated as an outpatient for one year for a squamous cell carcinoma of the face and a basal cell carcinoma of the neck.

Physical examination revealed the temperature to be 99.6°F.; pulse, 110; respirations, 30; blood pressure, 170/90 mm. Hg.

The patient was comatose and the neck was stiff. The pupils were dilated and fixed. There was an ulcer 2 by 2 cm. over the left mastoid area. The deep tendon reflexes were absent, but the plantar responses were normal.

An x-ray film of the chest was interpreted as "possible bronchopneumonia." The films of the skull showed no abnormality.

Type 3 pneumococcus was identified in the spinal fluid by capsular swelling.

Fifteen million units of crystalline penicillin dissolved in 2 L. of glucose in water were given in the eighteen hours the patient survived. He also received 12 million units of crystalline penicillin intramuscularly. At the onset of therapy he received 25 units of ACTH intravenously followed by 150 mg. of ACTH intramuscularly in divided doses.

Eight hours after admission the patient had a blood pressure of 70/50 mm. Hg. A constant infusion of l-nor-epinephrine was started and continued until his death. The rate was adjusted to maintain a systolic pressure over 100 and below 120. Shortly before his death one convulsion was noted. Cyanosis was evident even while breathing 100 per cent oxygen. Blood pressure and pulse became unobtainable and the patient died eighteen hours after his admission.

No autopsy was obtained.

Case xii. (WW 146645). A seventy-two year old Negro man was in coma when admitted to the hospital for the second time. His illness had begun one week before admission with malaise, fever, cough, pain in the neck, and disorientation progressing to coma on the day of admission.

On physical examination the temperature was 104°F.; pulse, 110; blood pressure, 160/90 mm. Hg. The pupils reacted to light and the fundus was normal. There were no physical signs of pneumonia, and the examination of the heart revealed no abnormalities. Aside from a stiff neck and slightly increased deep tendon reflex activity the neurologic examination was normal.

Table xii—Treatment

Day	Penicillin, Intravenously (millions of units)	ACTH, Intravenously (units)	Hydrocortisone, Intravenously (mg.)
0	30	60	60
1	30	60	160
2	30	60	100
3	30	40	100
4	30	60	90
5	30	60	90
6	15	60	75
7	15	60	75
8	15	60	45
9	10	60	0
10	0	40*	0

* Intramuscularly.

The diagnosis of pneumococcal meningitis was made upon identifying type 17 pneumococcus in the spinal fluid by capsular swelling. A blood culture also yielded type 17 pneumococcus.

For the first two days the patient appeared moribund and there was no decrease in fever or meningeal signs. A tracheostomy was performed on day 2 because it was believed that the patient was unable to handle his secretion adequately and a generalized convulsion was treated then with an anticonvulsant.

By day 2 there was no more fever and there was a slight increase in responsiveness to stimuli. On day 3 there was some increase in responsiveness although the patient still would not talk or follow commands. At this time he had gross muscular twitchings but no true convulsions. By day 4 the patient was alert and talking. Improvement to his normal status ensued on the next day. The penicillin was discontinued after ten days when he returned to his home cured for the second time of pneumococcal meningitis. (Table XII.)

RESULTS

Table XIII gives a résumé of the results in each patient. The recovery rate was 92 per cent, one of the patients dying eighteen hours after admission. In another patient, who died six months later of pneumonia and a brain tumor, no residual evidence of meningitis was found at autopsy. In the survivors no objective residual damage was detected by clinical examination at the time of discharge. One patient complained of dizziness; another, with a defect in the dura due to trauma, experienced a convulsion seven months after release from the hospital.

The median time for the length of fever was thirty-six hours in nine survivors. Two patients did not have fever. Three patients who were semi-comatose on admission became alert within thirty-six hours.

Side Reactions to ACTH. Most of the patients had glycosuria but it is not apparent from the records if the urine specimens were taken when the patients were receiving glucose in the infusion of penicillin. Glycosuria disappeared uniformly after recovery.

Hypokalemia appeared for two days in one patient who had experienced diarrhea before her admission and who received ACTH and potassium-free fluids. This was accompanied by an alkalosis, pH 7.52, but normal CO_2-combining power. It was believed, therefore, that this was an instance of a mixed respiratory and metabolic alkalosis—the respiratory alkalosis resulting from hyperventilation due to pneumonia. This patient also exhibited a salt-wasting syndrome thought to be cerebral in origin [*27*].

In one of the patients facial edema developed. When this was noted, the ACTH was discontinued. In one patient pyelonephritis developed due to Pseudomonas aeruginosa ten days after the discontinuation of ACTH. There was no tuberculosis. No one reported symptoms of peptic ulcer. Radiologic examination of the stomach was not carried out.

COMMENTS

The rate of recovery among these patients was higher than that reported previously among adults with pneumococcal meningitis, and residual damage was not present. The results are offered as evidence, therefore, to support the concept that ACTH and adrenal steroids are beneficial in the treatment of pneumococcal meningitis. This concept is based on the suppressive action exerted by these drugs in two potentially harmful features of pneumococcal infection: (1) inflammatory exudation, and (2) bacterial hypersensitivity.

In pneumococcal infections an inflammatory response is of little value to the host if a bactericidal drug is used. Wood and Smith [*28–30*] found in studies on the action of penicillin that leukocytes speed up the destruction of viable bacteria only if the bacteria are multiplying. That inflammation alone is an inadequate defense against invasion of the meninges by the pneumococcus is evident from the complete absence of recovery before sulfonamides were used and from the poor results obtained with these bacteriostatic agents [*14*]. Indeed, in pneumococcal meningitis the inflammatory response may increase morbidity as a result of the increased pressure of the purulent exudate before optimum antibacterial therapy is effective.

In addition to the deleterious action of the exudate directly on the host, inflammatory products can exert bacteriostatic effects on the pneumococcus which prevent the bactericidal action of penicillin. This has been demonstrated in experimental pneumonias by Wood and Smith [*28–30*] who observed that the rate of bacterial death was faster in the peripheral areas where the pneumococci multiply rapidly than in the central areas of the lesion which are rich in inflammatory cells. Aside from the bacteriostatic effect of the leukocytes it is also possible that they protect the intracellular pneumococci from contact with penicillin.

The effectiveness of ACTH and cortisone in

TABLE II

SUMMARY OF CASES TREATED FOR PNEUMOCOCCAL MENINGITIS WITH ACTH AND ADRENAL STEROIDS IN CONJUNCTION WITH PENICILLIN

Case No.	Age (yr.), Race, Sex	Duration of Illness Before Treatment (days)	Symptoms	Origin of Meningitis	Cerebrospinal Fluid Findings*							Duration of Fever	Duration of Coma	Blood Culture
					Day	OP	WBC	% Pmn	Prot	Sugar	Cult			
I (54720)	27, W, M		Fever, headache, vomiting, disorientation	Old skull fracture	0	550	20.0	90	Pell	20	− (Not typed)	8 hr.	None	Sterile
					1	250	1.8	88	192	71	−			
					4	130	0.06	3	56	48	−			
					7	95	0.02	60	32	54	−			
II (119951)	35, N, F	2–3	Coma	Pneumonia	0	260	0.6	90	....	25	+ (Type 6)	5 days	7 days	Type 6
					2	280	0.1	73	290	23	−			
					6	300	0.3	88	144	40	−			
					10	280	0.3	54	151	30	−			
					15	285	0.09	44	154	50	−			
					21	210	0.04	60	152	58	−			
					29	146	0.03	10	114	53	−			
III (66403)	36, N, F	2–4	Semicomatose	Mastoiditis	0	460	0.3	30	103	33	+ (Type 25)	12 hr.	1 day	Type 25
					7	160	0.1	24	56	65	−			
					14	145	0.02	20	50	60	−			
					21	.....	0.03	0	40	60	−			
IV (75590)	44, N, M	1	Fever, chills, convulsions, semicomatose	Dural defect	0	340	0.8	90	....	85	+ (Type 33)	24 hr.	36 hr.	Not performed
					1	490	11.2	95	620	28	+			
					5	.....	0.1	11	....	...	.....			
					13	.....	0.08	0	62	63	.....			
V (109226)	46, W, F	2–3	Comatose, convulsions, shock	Unknown	0	380	0.9	87	680	29	+	60 hr.	6 days	Type 14
					1	370	10.7	99	1180	38	−			
					4	140	2.3	22	464	40	−			
					7	180	0.01	0	150	97	−			
					20	100	0.08	0	137	61	−			
					27	95	0.01	0	84	...	−			
VI (McK VAH 64–126)	50, W, M	2	Fever, chills, headache, coma	Unknown	0	330	13.6	98	267	12	+ (Not typed)	36 hr.	5 days	Positive (not typed)
VII (70064)	55, N, F	1	Coma, convulsions	Sarcoma, left orbit	0	240	3.6	95	....	15	+ (Not typed)	None	6 days	Not performed
					1	365	5.6	98	....	...	−			
					4	140	0.7	35	220	56	−			
					6	230	0.3	80	90	67	−			
					7	275	0.03	..	....	45	−			
					8	180	0.01	0	....	77	−			
					17	.....	0.01	0	36	53	−			
VIII (111561)	56, M, W	1 (sudden onset of coma)	Coma	Sinusitis, pituitary tumor	0	>500*	13.3	90	620	11	+ (Type 7)	22 days	22 days	Type 7
					1	500	.....	..	1700	10	−			
					3	200	Many	88	600	62	−			
					6	190	0.2	90	264	70	−			
					12	190	0.07	90	124	50	−			
					28	.....	0	0	136	...	.....	..		
IX (136203)	64, N, F	2	Coma, convulsions, fever	? of sinusitis	0	450	0.8	76	400	16	+ (Type 12)	8 hr.	4 days	Type 12
					3	190	0.1	..	....	...	−			
					8	150	0.1	71	86	123	−			
					16	100	0.06	10	76	62	−			
X (146645)	72, N, M	2	Semicomatose	Unknown	0	400	10.6	90	190	30	+ (Type 17)	None	8 hr.	Type 17
					1	.....	0.6	58	23	36	.....			
					5	210	0.01	6	68	65	.....			
XI (99636)	73, W, M	Found unconscious	Coma	Otitis, pneumonia	0	280	0.3	84	830	13	+ (Type 3)	Died 18 hr.	.....	Type 3
XII (146645)	72, N, M	1	Coma	Unknown	0	>600	4.0	94	750	15	+	48 hr.	4 days	Type 17
					1	200	4.8	90	1500	14	−			
					4	65	8	37	37	600	−			

* OP = opening pressure in mm. of water.
% Pmn = per cent polymorphonuclears in spinal fluid.
Sugar = sugar in spinal fluid (mg./100 ml.).
— = sterile
Pell = pellicle.

WBC = white blood count in spinal fluid (× 1000).
Prot = Protein content of spinal fluid (mg./100 ml.).
Cult = culture of spinal fluid
+ = growth of pneumococci

suppressing the exudative reaction in pneumococcal infections has been demonstrated both experimentally and clinically. In experimental pneumococcal infections of the skin, Germuth, Ottinger and Oyama [*17*] observed that prior administration of cortisone reduced local inflammation. The lesions of the control rabbits contained numerous neutrophils, hemorrhages, edema, necrosis and thrombosis of small blood vessels, but in the infected skin of animals receiving cortisone leukocytes were sparse and the blood vessels normal. In clinical studies, Kass, Ingbar and Finland [*16*] found that ACTH administered after infection can reduce the inflammatory response despite the persistence of pneumococci in the tissues. In a patient with pneumococcal pneumonia treated with ACTH and no antibiotics they observed rapid resolution of the consolidation while pneumococci remained in both the sputum and blood. Evaluation of the anti-inflammatory effectiveness of steroids in the present cases is difficult because of the simultaneous use of penicillin, but the remarkable decrease in spinal fluid pressure, protein content and cell counts in certain patients (notably in Cases I and X) would not appear to be attributable to penicillin alone.

The importance of steroids in protecting the patient from the second postulated effect of pneumococcal infection—namely, bacterial hypersensitivity—is difficult to establish but is important to consider. The extreme vulnerability of the nervous system of sensitized individuals to local and generalized reactions from both bacterial and non-bacterial antigens has been demonstrated experimentally in the classic studies of Besredka [*31*] and clinically by various investigators who have injected streptococcal antigens and tuberculoprotein intrathecally [*32–34*]. These reactions, which have consisted of a marked outpouring of leukocytes and protein in the spinal fluid as well as nuchal rigidity, headache, high fever, convulsions and coma, are identical with the clinical manifestations of pneumococcal meningitis. Hypersensitivity to pneumococcal nucleoprotein is known to be widespread in the general population, and is of the delayed type elicited by a variety of bacterial antigens [*35*]. Because bacterial hypersensitivity of the delayed type has been inhibited by treatment with ACTH and steroid hormones, the possibility must be considered that their use in patients with pneumococcal infections might block a hypersensitivity reaction elicited both locally and systemically by the pneumococcal antigens even after the bacteria have been killed by penicillin [*36*].

Whether or not the benefits ascribed to adrenal steroids in pneumococcal meningitis will be borne out in future studies, it is becoming increasingly evident that the early fears related to their supposed harm in infection are for the most part unwarranted. Most of this apprehension was based on the lowered resistance to latent infections and their rapid dissemination observed after the unrealistic experimental and clinical use of massive doses of adrenal hormones for long periods of time [*37,38*]. The reports of numerous investigators have testified to the safety of adrenal hormones when given in doses which were clearly of benefit [*22–26,39–41*]. In each of these cases ACTH or cortisone was administered for acute infections over a period of time short enough to produce the desired clinical response without harm to the patient. The present study indicates that these hormones may be administered even to desperately ill patients with no increase in the incidence of untoward effects.

SUMMARY

1. Only one death occurred in twelve cases of pneumococcal meningitis in adults treated with ACTH and adrenal steroids in conjunction with penicillin.

2. These therapeutic results are discussed from the standpoint of the beneficial effect of ACTH and adrenal steroids in suppressing inflammation and hypersensitivity to the pneumococcus.

3. No serious deleterious effects of ACTH were noted.

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The Influence of Androgens, Estrogens and Related Steroids on Serum Lipids and Lipoproteins

*Observations in Hypogonadal and Normal Human Subjects**

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THERE are many diseases to which men are more vulnerable than women [*1*]. The most important of these is coronary atherosclerosis. The frequency with which the chief clinical manifestations of this disorder are found in men exceeds that in women severalfold [*2,3*]. This sex differential was recognized almost two centuries ago when William Heberden, in his treatise on "Pectoris Dolor," the classic description of the disease to which he gave the name "angina pectoris," wrote: "I have seen nearly a hundred people under this disorder, of which number there have been three women, and one boy 12 years old. All the rest were men, near, or past the fiftieth year of age."‡

Anatomic studies such as those described by Sjövall and Wihman [*4*], Ackerman, Dry and Edwards [*5*] and Schlesinger and Zoll [*6*] indicate an earlier and more extensive degree of coronary atherosclerosis in the male, although the sex difference is less impressive than is the case with respect to the clinical manifestations.

The concept of sex as a vulnerability factor in coronary atherosclerosis is lent further support by the observations of White [*7*], Gertler [*8*] and Hawke [*9*] that eunuchs manifest less extensive coronary atherosclerosis at autopsy than do noncastrated men, and by the studies of Wuest, Dry and Edwards [*10*] and Rivin and Dimitroff [*11*] indicating that otherwise healthy castrated women experience coronary atherosclerosis of a severity resembling that of men.

A sex difference in susceptibility to experimentally induced coronary atherosclerosis has been reported by Katz and co-workers who found that cholesterol feeding resulted in coronary atherosclerosis in roosters but not in the laying hen [*12*], and that prophylactic estrogen administration inhibited coronary but not aortic atherosclerosis in the cholesterol-fed chick [*13,14*]. Androgen administered with estrogen did not inhibit the prophylactic effect of the latter agent [*12*]. Studies of the influence of gonadal steroids on cholesterol-induced atherosclerosis in rabbits usually have been limited to the aorta and conflicting reports have appeared [*15,16*]. Mann has reported sex differences in the response of the serum cholesterol in rabbits fed cholesterol [*17,18*].

Studies of the influence of sex and gonadal steroids on serum lipids in humans have figured prominently in the approach to the problem of sex differences in coronary atherosclerosis. Serum cholesterol and phospholipid levels in

‡ Although Heberden's original publication appeared in 1772, the quotation is from his "Commentaries on the History and Cure of Diseases" published posthumously in London, 1802. (From Cardiac Classics by F. A. Willius and T. E. Keys, The C. V. Mosby Company, St. Louis, 1941.)

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women are reported to be similar to or higher than those in men of the same age group [*19–21*], to rise during pregnancy [*22*] and to vary with the menstrual cycle [*23*]. Lipid levels manifest different age trends in men and women [*24*]. Estrogens are reported to depress the cholesterol: phospholipid ratio in pre- and postmenopausal women [*25,26*] and in patients with coronary sclerosis [*27–37*] by increasing serum phospholipid concentrations and/or by depressing serum total cholesterol levels.

With the development of ultracentrifugal, electrophoretic and chemical fractionation methods, significant differences in the lipoprotein distribution in human serums attributable to sex or gonadal steroids are becoming clear.

Employing Cohn method-10 for fractionation, Russ, Eder and Barr [*29–32,38*] found that plasma from young women contained more "alpha" lipoprotein and less "beta" lipoprotein than that from men of comparable age. Their conclusions regarding the alpha and beta lipoproteins were based on the amount of cholesterol found in fractions IV + V + VI and I + III, respectively. Lewis, Green and Page [*39–41*] employing ultracentrifugation noted a similar sex difference with respect to the high density $-S_{1.21}$ 0–12 (alpha) and lower density $-S_{1.21}$ 25–40 (beta) lipoprotein concentrations in human serums. Jones et al. [*42*], utilizing Gofman's ultracentrifugal technic [*43*], reported significantly greater (tenfold) concentrations of S_f 10–100 (lower density) lipoproteins in serums from men in the age group thirty-one to forty years. DeLalla, Elliott and Gofman [*44*] reported significantly fewer low density lipoproteins (d = 1.05 gm./ml.) in healthy women than in men under forty years of age, and significantly greater concentrations of high density lipoproteins (d = 1.075 gm./ml. and d = 1.145 gm./ml.) in women than in men in the twenty to sixty year age group. Havel, Eder and Bragdon [*45*] found the concentrations of serum cholesterol and lipid phosphorus similar in both sexes, but men had more lipid in the lower density fractions and women more in the high density fractions. Nikkilä [*46*] and Antonini [*47*], using paper electrophoresis, found more serum cholesterol bound as beta lipoprotein in men below the age of forty, while women in the same age group had more cholesterol bound as alpha lipoprotein.

Significant differences between the serum lipoprotein distributions of eunuchs and of noncastrated men have been demonstrated [*48*].

Studies of the influence of gonadal steroids on serum lipoproteins followed as a natural consequence of the observations cited. Barr, Russ and Eder [*29–32,49*] reported an increase in cholesterol in Cohn fractions IV + V + VI and a decrease in fractions I + III in survivors of myocardial infarction treated with estrogens; the effect of androgen was noted to be opposite to that of estrogen [*30,32,49*]. On the other hand, Engelberg, Glass and Marcus [*50,51*] reported no change in the serum lipids or low density lipoproteins following the administration of estradiol for periods up to four months. A "moderate" rise in the S_f 12–100 lipoproteins was reported after testosterone administration. Robinson and co-workers [*37*], using paper electrophoresis, reported a significant reduction in the beta: alpha lipoprotein ratio in human subjects after one month of treatment with "mixed conjugated equine estrogens." Oliver and Boyd [*52*], also employing paper electrophoresis, reported increased alpha lipoprotein cholesterol, decreased beta cholesterol, serum cholesterol and C/P ratios following estrogen administration in "hypercholesterolemic" men with coronary artery disease. Opposite effects were noted following methyl testosterone administration.

We have utilized ultracentrifugation and a solvent density of 1.21 gm./ml. almost exclusively in our studies of the influence of gonadal and related steroids on serum lipids and lipoproteins. Preliminary reports have appeared [*53–55*].

SUBJECTS AND METHODS

While most of the observations reported to date from other laboratories have dealt with survivors from myocardial infarction, this report deals in the main with subjects in whom there was no clinically manifest coronary atherosclerosis or hypercholesterolemia. The age span of the subjects studied is great, and atherosclerosis was undoubtedly present in varying degree in many. Most of them were hypogonadal. A brief description of each subject may be found in the appendix.

The doses of gonadal steroids employed in this study elicited the expected secondary sex responses. These were often subjective only, and in only one instance forced alterations in individual study plans. In every instance it was possible to observe significant alterations in the serum lipoprotein spectrum prior to their development. In one male subject (A. T.) stilbestrol, 3 mg./day, caused sufficient lack of potentia to cause him to discontinue the drug surreptitiously. This defection was quickly detected in the lipoprotein pat-

tern of the next serum sample. Occasionally no secondary sex effects were noted (P. H., G. M., R. M., M. R., G. T.). In one subject (M. P.) mild congestive failure with angina developed during nortestosterone administration. This responded well to sodium restriction.

The studies reported here were culled from and are representative of a four-year experience involving more than sixty subjects, some of whom have been studied for eighteen months or longer. The following steroids have been utilized:

ANDROGENS (oral)	ESTROGENS (oral)
Methyl testosterone*†	Diethylstilbestrol**
19-Nortestosterone‡	Conjugated equine estrogens (premarin®)¶
17-Methyl-19-nortestosterone§	Estrone sulfate¶
17-Ethyl-19-nortestosterone‖	Equilin sulfate¶
Testosterone (sublingual)¶	Mixture of non-ketonic estrogens as sulfate¶
(parenteral)	(parenteral)
Testosterone propionate (aqueous)*	Estradiol benzoate (in oil)††
Testosterone isobutyrate (aqueous)*	Estradiol (aqueous)††
	OTHER STEROIDS
Testosterone propionate (in oil)†	Progesterone (in oil) (parenteral)*
Testosterone cyclopentylpropionate (in oil)†	Progesterone (sublingual)¶
19-Nortestosterone propionate (in oil)‡	Allopregnanolone (oral)‡‡

* Ciba Pharmaceutical Products, Inc.
† The Upjohn Company.
‡ Smith, Kline and French Laboratories.
§ Organon, Inc.
‖ G. D. Searle & Co.
¶ Ayerst Laboratories.
** Winthrop Laboratories.
†† Schering Corporation.
‡‡ The Glidden Company.

Ultracentrifugation of postabsorptive serum was carried out according to the method of Green, Lewis and Page [*41*]. Preparative centrifugation was accomplished in the Spinco Model L ultracentrifuge at 17°c. for thirteen hours in the 30.2 rotor at 30,000 r.p.m. (79,420 × gravity), solvent density 1.21 gm./ml. (Repeated determination of the density of the infranatant after preparative centrifugation has consistently revealed a specific gravity of 1.22.)

It has been suggested recently that this method of preparative centrifugation results in incomplete cholesterol flotation [*45*]. We have analyzed supernatant and infranatant fractions after preparative centrifugation by this method, and in serums from apparently healthy subjects note less than 2.7 per cent of the total cholesterol concentration of native serum remaining in the bottom fractions [*56*]. The technical consistency of removal of the top fraction by suction, after preparative centrifugation, is within 3.3 per cent in this laboratory.

Analytic ultracentrifugation was accomplished in the Model E ultracentrifuge utilizing an "A" rotor at 52,640 r.p.m. (215,000 × gravity), 26.0 ± 0.5°c. Analytic ultracentrifugation was repeated at a lower concentration when necessary to provide $-S_{1.21}$ 25–40 or 25–70 peaks free of obscuring refractive bands.

All planimetry in this study was carried out in triplicate by the same observer. High density lipoprotein peak areas were usually determined in the patterns obtained after centrifugation at a five-fold concentration ($5C_0$) and lower density lipoprotein peak areas usually after centrifugation at $2C_0$ or $3C_0$. This permitted planimetry of maximum peak areas without extrapolation or other means of approximation. Strict attention was not paid to minor variations in flotation rates of the major lipoproteins nor was correction for Johnston-Ogston effects attempted. Conversion of area in square millimeters to milligrams per cent lipoprotein was accomplished utilizing a formulation presented at the Technical Symposium of the Technical Group, Committee on Lipoproteins and Atherosclerosis, National Advisory Heart Council, 1952.

The value for the specific refractive increment for lipoproteins in the medium used in this study is 0.00168.*

Lipoproteins separated by this method are designated in $-S_{1.21}$ units of flotation. The class $-S_{1.21}$ 0–12 probably represents, in health, most of the lipoproteins found in Cohn fractions IV + V + VI and the alpha lipoproteins as determined by electrophoresis, while the lipoprotein class $-S_{1.21}$ 25–40 corresponds to the lipoproteins designated by Gofman as S_f 0–12 and probably resembles those found in healthy subjects in Cohn fractions I + III or the beta lipoproteins as determined electrophoretically [*57*]. The $-S_{1.21}$ 40–70 lipoprotein class corresponds to the S_f 12–20 class.

For purposes of clarity and brevity the term "high density" is used to designate the $-S_{1.21}$ 0–12 (alpha) lipoprotein fraction, while "lower density" is used to designate the $-S_{1.21}$ 25–40 (S_f 0–12 or beta) lipoprotein fraction.

In this study each subject served as his own control, with observations prior to and/or after medication was stopped. Uniformity of technic with respect to analytical ultracentrifuge pattern integration was carefully maintained. These factors insure the validity of results with regard to relative changes in lipoprotein spectrums brought about by the agent administered.

In the charts accompanying this paper the alpha/beta lipoprotein ratio is designated as the $-S_{1.21}$ 0–12/

* This value was obtained from Dr. Lena Lewis. It is derived from the observation that the peak area of a solution of beta lipoprotein at d = 1.063 was 1.09 × that of the same solution at solvent density 1.21. Therefore multiplying "n" for solvents of density 1.063 gm./ml. (generally given as .00154) by 1.09 yields the value .00168.

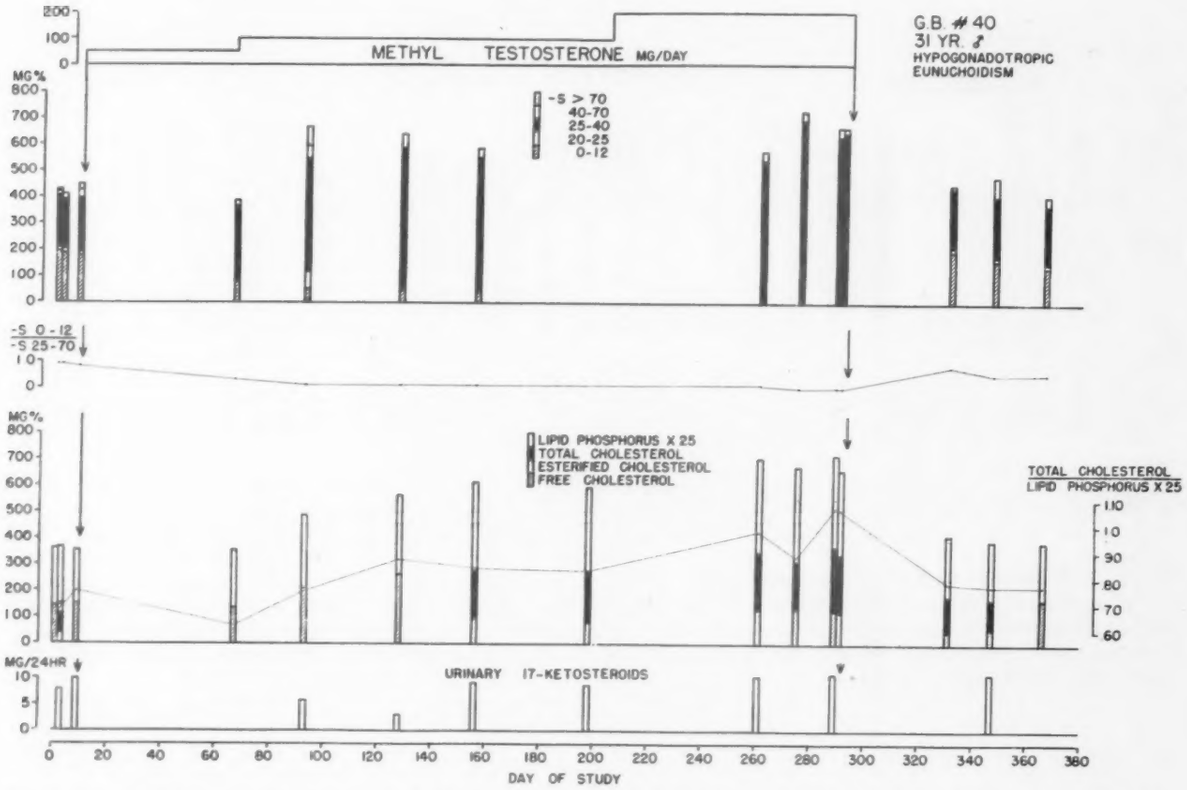

FIG. 1. G. B., thirty-one year old eunuchoidal man. The serum high density ($-S_{1.21}$ 0–12) lipoprotein concentration was markedly reduced, and eventually ultracentrifugal evidence of these lipoproteins was eliminated, after prolonged administration of methyl testosterone. Urinary 17-ketosteroid excretion did not change significantly. Hypercholesterolemia developed as the high density lipoprotein concentration fell.

$-S_{1.21}$ 25–70 ratio, in order to avoid any inaccuracies inherent in the planimetry of the $-S_{1.21}$ 25–40 and $-S_{1.21}$ 40–70 areas independently.

Total and unesterified cholesterol were determined by the method of Sperry and Webb [*58*] and lipid phosphorus by a modification [*59*] of the method of Youngburg [*60*]. The factor of 25 was used to convert lipid phosphorus to phospholipid.

Urinary gonadotropin assays were carried out essentially according to the method of Klinefelter, Albright and Griswold [*61*]. Cellophane dialysis was used when the gonadotropin level was expected to be low and in these instances the assay is reported in terms of 6.5, 13, 26 and 52 mouse units (m.u.) per day (normal values $>6.5 < 26$ m.u./24 hours). When dialysis was not required the unitage is in terms of 48, 96, 192, and 384 mouse units per 24 hours (normal values <96 m.u./24 hours).

Urinary neutral 17-ketosteroids were determined utilizing the Zimmerman reaction. Initially, acid hydrolysis, extraction with carbon tetrachloride and a modified Zimmerman reaction were employed according to the technic outlined by Holtorff and Koch [*62*]. In later studies the technic proposed by Klendshoj, Feldstein and Sprague [*63*] was employed. By either of these technics, results greater than 16 mg. per 24 hours are abnormally high and less than 6 mg. per 24 hours are abnormally low for adult men or women.

RESULTS

High density $-S_{1.21}$ 0–12 lipoprotein concentrations changed promptly and significantly when gonadal steroids were administered. Only when small doses and relatively weak agents were employed were rare exceptions to this rule noted. Androgens reduced high density lipoprotein concentrations (Figs. 1, 2 and 3); estrogens increased them (Figs. 3 and 4). In one subject (G. B.) prolonged androgen administration eliminated all centrifugal evidence of high density lipoproteins. The values for the ratios $-S_{1.21}\frac{0\text{–}12}{25\text{–}40}\left(\frac{\text{alpha}}{\text{beta}}\right)$, $-S_{1.21}\frac{0\text{–}12}{25\text{–}70}$ and $\frac{-S_{1.21}\ 0\text{–}12}{\text{serum total cholesterol}}$ changed with the high density $-S_{1.21}$ 0–12 lipoproteins. All of these changes were frequently noted within forty-

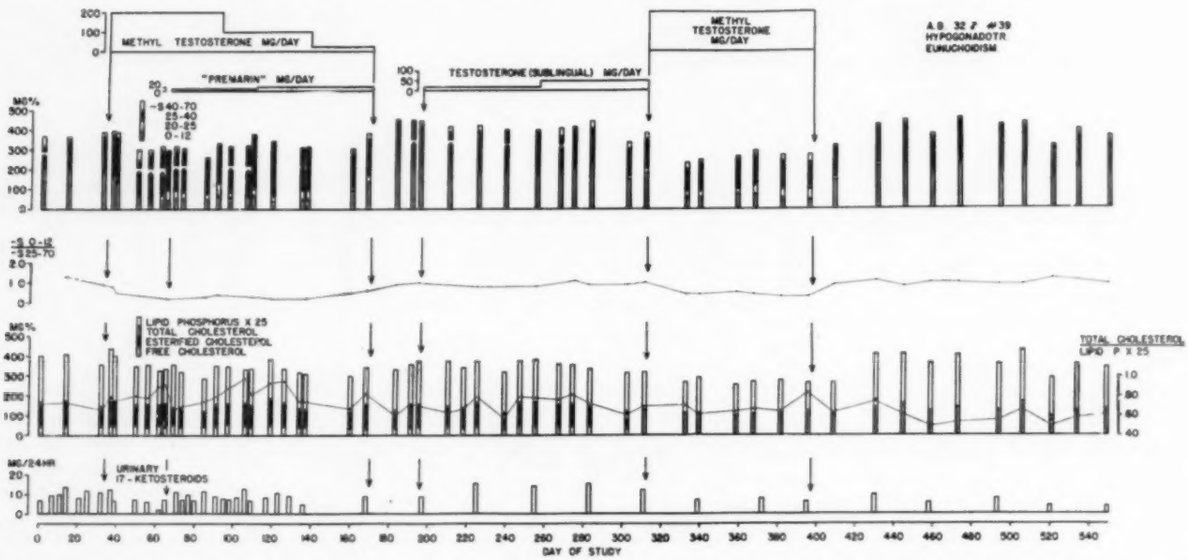

FIG. 2. A. B., thirty-two year old eunuchoidal man. Marked reduction in serum high density ($-S_{1.21}$ 0–12) lipoprotein and phospholipid concentrations resulted from the administration of methyl testosterone. The combined daily administration of methyl testosterone 25 mg. and premarin 20 mg. resulted in the androgenic effect predominating. Urinary 17-ketosteroid excretion was greatest during sublingual testosterone administration, although this agent was relatively ineffective in altering lipoproteins.

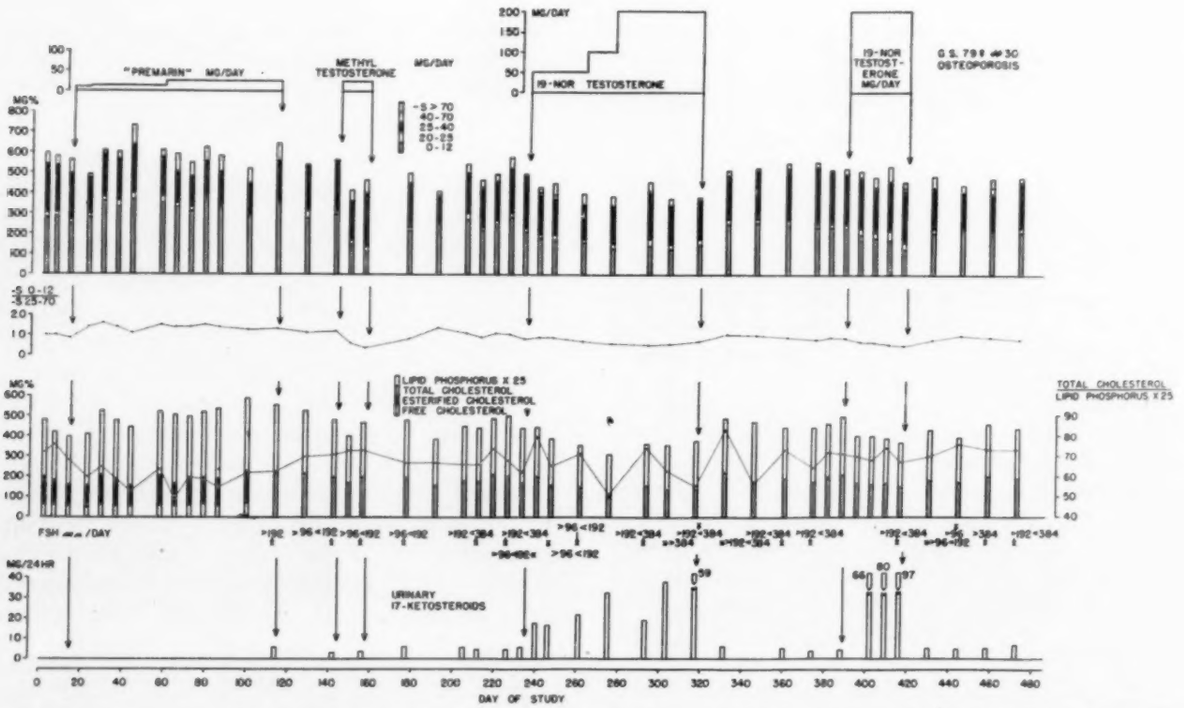

FIG. 3. G. S., seventy-nine year old woman with osteoporosis. Androgen administration resulted in reduction in serum high density ($-S_{1.21}$ 0–12) lipoprotein concentrations. Premarin administration was associated with modest increase in high density lipoprotein and phospholipid concentrations. Marked differences in urinary 17-ketosteroid excretion are apparent with respect to methyl testosterone and 19-nortestosterone administration. Androgen administration was associated with significant reduction in high density lipoprotein concentrations, although the urinary excretion of gonadotropin remained at high levels.

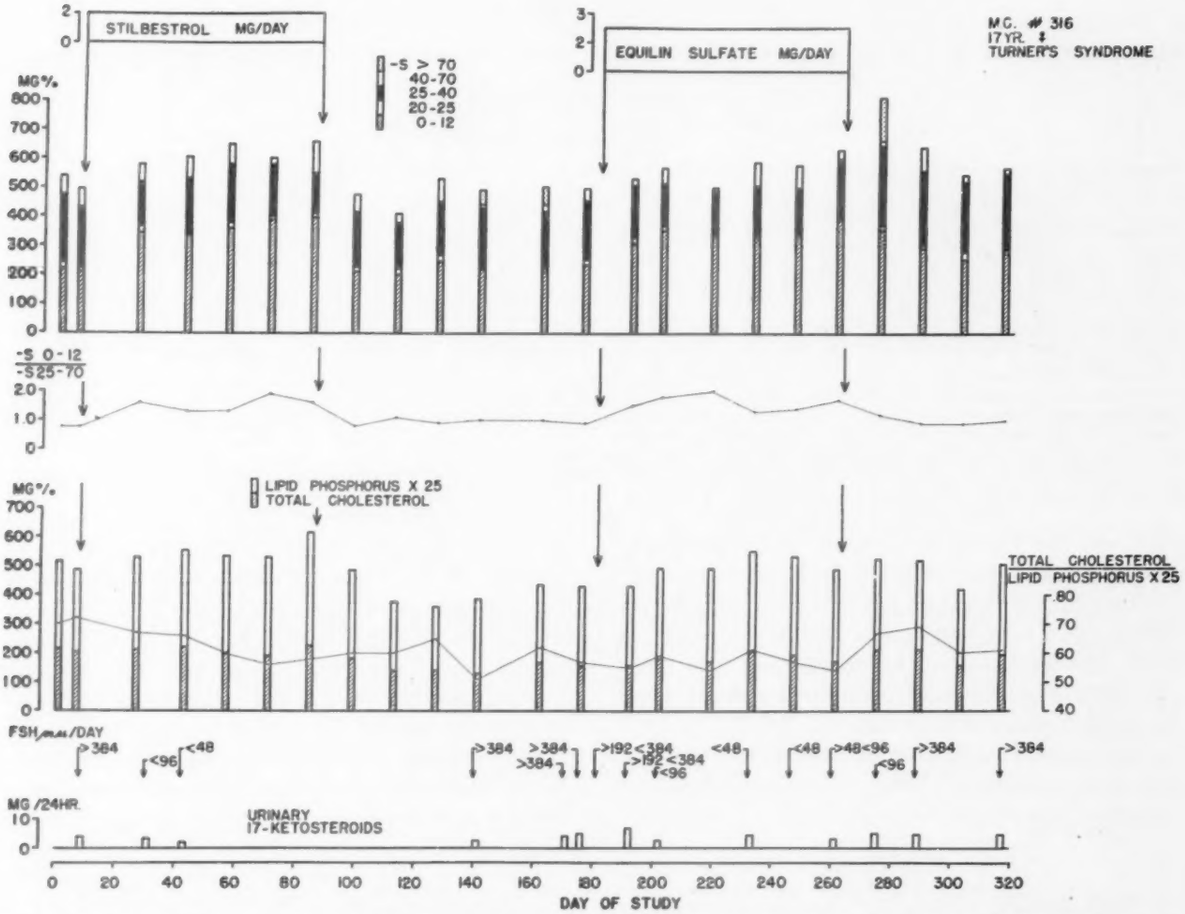

FIG. 4. M. C., sixteen year old girl with Turner's syndrome. Both stilbestrol and equilin sulfate administration were associated with increases in serum high density ($-S_{1.21}$ 0–12) lipoprotein and phospholipid concentrations. An appreciable increase in high density lipoproteins was noted in the first analysis after equilin sulfate administration began, and at a time when urinary gonadotropin excretion was still >192 mouse units/day.

eight hours of beginning therapy, but when therapy was discontinued it was often two or more weeks before control values were once again attained.

The data in Table I are representative of our experience with gonadal steroid administration. It is noteworthy that lower density $-S_{1.21}$ 25–40 and 25–70 lipoprotein concentrations tended to vary in a direction opposite to that of the high density $-S_{1.21}$ 0–12 lipoproteins when androgens were administered, but little change in lower density lipoprotein concentrations occurred when estrogens were administered, even though appreciable increases in high density $-S_{1.21}$ 0–12 lipoproteins occurred. However, the response of the lower density $-S_{1.21}$ 24–40 and 25–70 lipoprotein concentrations to the administration and withdrawal of gonadal steroids showed considerable variation among individual subjects which could not be accounted for or predicted on the basis of age, sex, endocrine status or other clinical or related laboratory information.

The responses of the serum lipids, lipoproteins and lipoprotein ratios to the administration or withdrawal of gonadal steroids in thirty-one subjects is tabulated in Tables II to VI. Tables II to V summarize the serum lipid and lipoprotein changes occurring when androgens were administered or discontinued a total of fifty-five times. Table VI indicates the changes noted when estrogens were administered or discontinued a total of twenty-nine times.

The over-all experience with respect to serum cholesterol and phospholipids indicated (Table I) that while serum cholesterol was little changed by gonadal steroids, serum phospholipids tended to fall when androgens were administered and to rise when estrogens were administered. (Figs.

TABLE I

RELATIVE CHANGE (PER CENT ± S.E.) INDUCED IN SERUM LIPIDS AND LIPOPROTEINS BY GONADAL STEROIDS *

$$(\%\ \text{change} = \frac{\text{mean value during Rx}}{\text{mean value before Rx}} \times 100)$$

Steroid	Cholesterol	Phospholipid	C/P Ratio	$-S_{1.21}$ 0–12	$-S_{1.21}$ 25–40	$-S_{1.21}$ 25–70	$\frac{-S_{1.21}\ 0\text{–}12}{25\text{–}40}$	$\frac{-S_{1.21}\ 0\text{–}12}{25\text{–}70}$	$\frac{-S_{1.21}\ 0\text{–}12}{\text{Total Cholesterol}}$	No. Subjects	No. of Courses of Steroid
Androgen†	101 ± 6	93 ± 4	107 ± 2	47 ± 4	134 ± 10	133 ± 9	40 ± 3	40 ± 3	50 ± 4	18	27
Estrogen‡	106 ± 4	116 ± 3	91 ± 3	135 ± 5	94 ± 5	100 ± 5	150 ± 10	142 ± 10	131 ± 6	12	17

* Many of the subjects included in this tabulation received only small doses of steroid. The changes presented, therefore, are not necessarily maximal, and statistical significance is not indicated in the table. Only the effects of steroid administration are dealt with in this table, in contrast to Tables II to VI in which the effects of cessation of therapy are also listed.

† Methyl testosterone, 19-nortestosterone, 17-methyl nortestosterone, 17-ethyl nortestosterone.

‡ Stilbestrol, equilin sulfate, premarin, estrone sulfate, nonketonic estrogens.

2, 3 and 4.) Individual responses differed however, as may be noted in Tables II to VI.

Allopregnanolone administration was without effect on the serum lipids and lipoproteins in four subjects. Oral progesterone produced significant reduction in high density lipoprotein concentrations in one subject (M. H.), while intramuscular progesterone produced equivocal effects in another (W. W.). Further studies with progesterone are under way.

COMMENTS

High density $-S_{1.21}$ 0–12 lipoprotein concentrations are highly sensitive to change induced by gonadal steroid administration. "Physiologic" (i.e., small) doses of steroids suffice, and the high density lipoprotein response is consistent and prompt. This is also true of the ratios expressing the relation of high density to lower density lipoprotein concentrations, or the relation of high density lipoprotein concentration to that of serum total cholesterol, since the change in high density lipoproteins was always of sufficient magnitude to determine this relationship, regardless of the direction of change of the other serum lipids or lipoproteins. This consistency of response makes it feasible to relate the change in the concentrations of lower density lipoproteins, cholesterol and phospholipids to that of the high density lipoproteins.

Inspection of Tables II to V reveals that when androgens were administered or discontinued, significant decrements or increments in high density lipoprotein concentrations occurred fifty-two times in fifty-five observations. Lower density lipoprotein concentrations changed in the opposite direction twenty-nine times, underwent no significant change sixteen times, and changed in the same direction as the high density lipoproteins seven times. (Table VII.) In other words, 87 per cent of the time the lower density lipoproteins either varied inversely or not at all with respect to high density lipoproteins. Keeping this in mind it is not surprising to note in Table VIII that, in relation to androgen therapy, serum cholesterol and phospholipid concentrations underwent either no change or varied in the same direction as the high density lipoproteins 85 per cent $\left(\frac{22 + 22}{52} \times 100 \text{ for cholesterol}\right)$ to 90 per cent $\left(\frac{21 + 26}{52} \times 100 \text{ for phospholipid}\right)$ of the time. No appreciable change in serum cholesterol and phospholipid levels would be anticipated when the two major lipoprotein fractions vary inversely; but the serum cholesterol and phospholipid levels would be expected to change in the same direction as the high density lipoprotein levels when little or no change is noted in the lower density lipoproteins. It is recognized, of course, that the quantitative aspects of these relationships are modified somewhat by the known qualitative differences in the lipid composition of these major lipoprotein fractions [*45*] and, furthermore, that the demonstration of this phenomenon does not establish a cause and effect relationship, nor determine which is the primary and which the secondary phenomenon.

Inspection of Table V reveals that when estrogens were administered or discontinued, significant increments or decrements in high density lipoprotein concentrations occurred twenty-six

TABLE II

EFFECTS OF METHYL TESTOSTERONE ON SERUM LIPIDS AND LIPOPROTEINS

Subject	Cholesterol	Phospholipid	C/P Ratio	$-S_{1.21}$ 0–12 (alpha)	$-S_{1.21}$ 25–40 (beta)	$-S_{1.21}$ 25–70	$-S_{1.21}\frac{0\text{–}12}{25\text{–}40}$	$-S_{1.21}\frac{0\text{–}12}{25\text{–}70}$	$\frac{-S_{1.21}\ 0\text{–}12}{\text{Total Cholesterol}}$	Therapy
A. B. 1	134 2	167 10	0.82 .05	59 10	161 13	172 11	0.37 .06	0.35 .05	0.44 .08	75 mg./day for 9 months, then 200 mg./day for 3 months
2	166 6 ↑ .01	206 13 ↑ .05	0.82 .07 ns	174 9 ↑ .001	161 6 ns	172 7 ns	1.09 .08 ↑ .001	1.02 .07 ↑ .001	1.06 .08 ↑ .001	Stop above Rx; no Rx for 3 months
3	158 9 ns	197 10 ns	0.81 .03 ns	79 17 ↓ .001	219 12 ↑ .01	231 12 ↑ .01	0.37 .08 ↓ .001	0.35 .09 ↓ .001	0.49 .08 ↓ .001	200 mg./day, then 100 mg./day for a total of 103 days
4	149 7 ns	204 4 ns	0.73 .03 ns	192 9 ↑ .001	183 10 ↓ .05	206 9 ns	1.06 .05 ↑ .001	0.94 .05 ↑ .001	1.30 .06 ↑ .001	Stop above Rx; no methyl testosterone for 6 months
5	109 3 ↓ .001	161 5 ↓ .01	0.68 .03 ns	65 5 ↓ .001	143 6 ↓ .01	167 6 ↓ .01	0.45 .04 ↓ .001	0.39 .03 ↓ .001	0.60 .06 ↓ .001	200 mg./day for 85 days
6	136 8 ↑ .01	240 8 ↑ .001	0.57 .02 ↓ .05	198 8 ↑ .001	186 10 ↑ .01	203 8 ↑ .01	1.07 .05 ↑ .001	0.98 .04 ↑ .001	1.46 .07 ↑ .001	Stop above Rx
J. P. 1	295 10	319 14	0.93 .06	103 14	421 49	479 49	0.25 .04	0.22 .04	0.35 .03	50–200 mg./day for 78 days
2	243 11 ↓ .01	293 13 ns	0.83 .04 ns	212 8 ↑ .001	278 8 ↓ .05	353 15 ↓ .05	0.77 .04 ↑ .001	0.61 .04 ↑ .001	0.87 .03 ↑ .001	Stop Rx; no Rx for 6 months
3	227 10 ns	286 8 ns	0.80 .03 ns	125 12 ↓ .001	284 15 ns	335 11 ns	0.44 .04 ↓ .001	0.37 .03 ↓ .001	0.55 .05 ↓ .001	50 mg./day for 6 months
4	247 23 ns	312 8 ns	0.80 .10 ns	261 23 ↑ .001	348 8 ↑ .01	395 11 ↑ .01	0.75 .05 ↑ .01	0.66 .04 ↑ .001	1.07 .12 ↑ .001	Stop above Rx
G. B. 1	147 3	213 5	0.69 .03	185 5	200 4	220 6	0.93 .03	0.84 .04	1.25 .06	No Rx for 1 year
2	342 10 ↑ .001	347 8 ↑ .001	0.99 .04 ↑ .01	1 1 ↓ .001	625 33 ↑ .001	655 32 ↑ .001	0.00 .00 ↓ .001	0.00 .00 ↓ .001	0.01 .01 ↓ .001	50–200 mg./day for 281 days
3	174 3 ↓ .001	225 4 ↓ .001	0.77 .01 ↓ .01	163 18 ↑ .001	225 3 ↓ .001	267 21 ↓ .001	0.73 .09 ↑ .001	0.62 .10 ↑ .01	0.95 .07 ↑ .001	Stop above Rx
H. B. 1	150 10	225 8	0.67 .07	227 15	123 10	157 9	1.87 .27	1.44 .02	1.52 .20	No Rx for 3 years
2	145 8 ns	219 5 ns	0.66 .03 ns	85 9 ↓ .01	243 5 ↑ .001	316 24 ↑ .01	0.35 .04 ↓ .01	0.27 .02 ↓ .001	0.58 .03 ↓ .01	200 mg./day for 5 months
G. M. 1	208 17	256 4	0.82 .02	165 4	257 7	291 7	0.64 .03	0.57 .02	0.79 .03	No Rx
2	321 41 ↑ .001	354 19 ↑ .01	0.91 .01 ↑ .001	65 3 ↓ .001	438 53 ↑ .01	511 51 ↑ .01	0.16 .02 ↓ .001	0.13 .01 ↓ .001	0.21 .02 ↓ .01	200 mg./day for 56 days
3	200 3 ↓ .001	256 5 ↓ .01	0.78 .01 ↓ .001	209 2 ↑ .001	220 2 ↓ .01	309 10 ↓ .01	0.95 .02 ↑ .001	0.67 .02 ↑ .001	1.05 .02 ↑ .001	Stop above Rx
R. M. 1	270 7	313 8	0.86 .01	183 7	266 14	348 12	0.70 .06	0.53 .03	0.68 .03	No Rx
2	185 4 ↓ .001	256 7 ↓ .001	0.72 .01 ↓ .001	75 2 ↓ .001	281 4 ns	384 8 ↑ .05	0.27 .01 ↓ .001	0.20 .01 ↓ .001	0.41 .01 ↓ .001	200 mg./day for 24 days
3	215 7 ↑ .01	267 8 ns	0.80 .02 ↑ .001	220 9 ↑ .001	232 11 ↓ .01	306 14 ↓ .001	0.96 .07 ↑ .01	0.73 .05 ↑ .001	1.03 .08 ↑ .001	Stop above Rx
J. M. 1	164 17	249 10	0.66 .04	245 31	133 14	140 8	1.84 .14	1.75 .20	1.49 .05	No Rx for 15 months
2	176 9 ns	221 7 ↓ .05	0.79 .03 ↑ .02	103 7 ↓ .001	240 15 ↑ .001	281 14 ↑ .001	0.44 .03 ↓ .001	0.37 .02 ↓ .001	0.60 .06 ↓ .001	100 mg./day for 15 months, 200 mg./day for 10 months
3	207 3 ↑ .01	283 11 ↑ .001	0.73 .02 ns	352 15 ↑ .001	181 5 ↓ .01	210 6 ↓ .001	1.95 .09 ↑ .001	1.68 .06 ↑ .001	1.70 .07 ↑ .001	Stop above Rx
Z. D. 1	197 6	242 6	0.81 .01	109 7	343 27	369 29	0.33 .04	0.30 .03	0.55 .03	25 mg./day for 56 days
2	183 12 ns	275 11 ↑ .05	0.67 .05 ↓ .05	195 8 ↑ .001	251 19 ↓ .05	275 23 ↓ .05	0.79 .05 ↑ .001	0.72 .05 ↑ .001	1.09 .10 ↑ .01	Stop Rx
M. R. 1	255 13	340 13	0.75 .06	343 17	252 16	279 22	1.37 .09	1.25 .11	1.35 .08	No Rx for 2 months
2	196 2 ↓ .01	269 14 ↓ .02	0.73 .04 ns	189 15 ↓ .01	330 23 ↑ .05	348 21 ns	0.58 .08 ↓ .01	0.55 .07 ↓ .01	0.97 .09 ↓ .05	30 mg./day for 47 days
3	227 6 ↑ .001	321 8 ↑ .02	0.71 .02 ns	315 12 ↑ .001	269 6 ↓ .05	291 6 ↓ .05	1.18 .07 ↑ .001	1.09 .05 ↑ .001	1.39 .07 ↑ .01	Stop above Rx
G. S. 1	183 15	251 19	0.73 .00	127 17	229 37	289 41	0.59 .17	0.46 .12	0.71 .15	25 mg./day for 14 days
2	186 8 ns	277 6 ns	0.67 .02 ↓ .05	246 15 ↑ .001	215 11 ns	250 9 ns	1.16 .10 ↑ .05	0.99 .06 ↑ .01	1.32 .06 ↑ .02	Stop Rx
P. H. 1	197 11	274 2	0.72 .04	210 6	234 13	270 15	0.90 .02	0.78 .05	1.01 .03	No Rx
2	194 11 ns	229 22 ↓ .01	0.85 .04 ns	93 8 ↓ .001	275 29 ns	312 19 ns	0.35 .06 ↓ .001	0.30 .04 ↓ .01	0.47 .03 ↓ .001	75 mg./day for 27 days
3	200 1 ns	289 12 ↑ .02	0.70 .03 ↓ .05	242 7 ↑ .001	261 7 ns	320 19 ns	0.93 .04 ↑ .01	0.76 .05 ↑ .01	1.21 .03 ↑ .001	Stop Rx

NOTE: Lipids and lipoproteins in mg. per cent ± S.E. Significance determined by t test; arrow indicates direction of change. ns = not significant.

TABLE III

EFFECTS OF 19-NORTESTOSTERONE ON SERUM LIPIDS AND LIPOPROTEINS

Subject	Cholesterol	Phospholipid	C/P Ratio	$-S_{1.21}$ 0–12 (alpha)	$-S_{1.21}$ 25–40 (beta)	$-S_{1.21}$ 25–70	$-S_{1.21}\frac{0\text{–}12}{25\text{–}40}$	$-S_{1.21}\frac{0\text{–}12}{25\text{–}70}$	$\frac{-S_{1.21}\ 0\text{–}12}{\text{Total Cholesterol}}$	Therapy
G. T. 1	173 6	287 3	0.60 .01	196 6	123 6	172 4	1.61 .10	1.14 .02	1.13 .03	No Rx
2	154 7 ns	232 15 ↓ .05	0.67 .01 ↑ .05	102 5 ↓ .001	168 12 ↑ .05	209 14 ns	0.61 .01 ↓ .001	0.49 .02 ↓ .001	0.67 .01 ↓ .001	200 mg./day for 21 days
3	147 10 ns	242 8 ns	0.61 .04 ns	132 8 ↑ .05	135 7 ns	173 7 ns	0.98 .01 ↑ .001	0.76 .02 ↑ .001	0.90 .08 ns	Stop above Rx
M. P. 1	216 11	317 4	0.68 .03	248 6	278 24	335 21	0.93 .08	0.75 .04	1.17 .08	No Rx for 4½ months
2	208 12 ns	280 5 ↓ .001	0.74 .03 ns	178 7 ↓ .001	270 23 ns	323 27 ns	0.68 .04 ↓ .02	0.56 .02 ↓ .01	0.88 .06 ↓ .02	100 mg./day for 12 weeks
3	209 12 ns	294 16 ns	0.71 .04 ns	233 10 ↑ .01	312 5 ns	339 7 ns	0.75 .02 ns	0.69 .02 ↑ .01	1.13 .06 ↑ .05	Stop above Rx
L. R. 1	191 5	232 3	0.82 .02	210 5	192 10	222 7	1.10 .06	0.95 .02	1.10 .02	Control
2	177 3 ↓ .05	206 3 ↓ .001	0.86 .02 ns	149 7 ↓ .001	199 1 ns	239 5 ns	0.75 .04 ↓ .01	0.63 .04 ↓ .001	0.84 .04 ↓ .001	50–200 mg./day for 4 weeks
3	213 5 ↑ .001	290 8 ↑ .001	0.73 .01 ↓ .001	336 15 ↑ .001	196 6 ns	228 8 ns	1.72 .08 ↑ .001	1.48 .06 ↑ .001	1.57 .04 ↑ .001	Stop above Rx
G. S. 2	186 8	277 6	0.67 .02	246 15	215 11	250 9	1.16 .10	0.99 .06	1.32 .06	No Rx for 10 weeks
3	147 11 ↓ .02	223 7 ↓ .001	0.66 .04 ns	149 8 ↓ .001	193 7 ns	225 10 ns	0.78 .06 ↓ .01	0.68 .06 ↓ .01	1.03 .05 ↓ .01	50–200 mg./day for 12 weeks
4	191 8 ↑ .01	274 7 ↑ .001	0.70 .11 ns	241 4 ↑ .001	247 9 ↑ .001	269 10 ↑ .01	0.99 .05 ↑ .02	0.90 .05 ↑ .02	1.27 .06 ↑ .02	Stop above Rx; no Rx for 10 weeks
5	159 4 ↓ .01	229 4 ↓ .001	0.69 .02 ns	167 15 ↓ .01	243 6 ns	286 10 ns	0.69 .07 ↓ .01	0.58 .05 ↓ .01	0.99 .07 ↓ .05	200 mg./day for 4 weeks
6	181 5 ↑ .02	247 8 ns	0.73 .01 ns	207 4 ↑ .05	198 11 ↓ .02	237 12 ↓ .02	1.05 .06 ↑ .01	0.88 .04 ↑ .01	1.15 .05 ns	Stop above Rx
S. B. 1	197 9	291 5	0.67 .02	258 13	205 11	239 11	1.28 .08	1.09 .08	1.31 .03	No endocrine Rx for 10 months
2	190 5 ns	224 6 ↓ .001	0.85 .01 ↑ .001	156 7 ↓ .001	244 6 ↑ .02	276 6 ↑ .02	0.64 .02 ↓ .001	0.56 .02 ↓ .001	0.82 .02 ↓ .001	100 mg./day for 11 weeks
3	209 5 ↑ .02	272 5 ↑ .001	0.77 .02 ↓ .01	252 17 ↑ .001	231 5 ns	256 6 ↓ .05	1.10 .09 ↑ .001	0.99 .06 ↑ .001	1.21 .08 ↑ .001	Stop above Rx
A. B. 6	136 8	240 8	0.57 .02	198 8	186 10	203 8	1.07 .05	0.98 .04	1.46 .07	No Rx for 5 months
7	145 5 ns	217 4 ↓ .05	0.67 .03 ↑ .05	176 5 ↓ .05	210 9 ns	236 11 ↑ .05	0.84 .03 ↓ .01	0.75 .02 ↓ .001	1.22 .06 ↓ .05	100 mg./day for 56 days then 200 mg./day for 28 days
8	136 7 ns	205 6 ns	0.66 .02 ns	197 6 ↑ .05	165 5 ↓ .01	181 5 ↓ .01	1.20 .08 ↑ .01	1.09 .06 ↑ .01	1.46 .11 ns	Stop above Rx

NOTE: Lipids and lipoproteins in mg. per cent ± S. E. Significance determined by t test; arrow indicates direction of change.

TABLE IV

EFFECTS OF 17-METHYL NORTESTOSTERONE AND 17-ETHYL NORTESTOSTERONE ON SERUM LIPIDS AND LIPOPROTEINS

Subject	Cholesterol	Phospholipid	C/P Ratio	$-S_{1.21}$ 0–12	$-S_{1.21}$ 25–40	$-S_{1.21}$ 25–70	$-S_{1.21}\frac{0\text{–}12}{25\text{–}40}$	$-S_{1.21}\frac{0\text{–}12}{25\text{–}70}$	$\frac{-S_{1.21}\ 0\text{–}12}{\text{Total Cholesterol}}$	Therapy
M. H. 1	205 8	246 8	0.84 .03	204 8	236 16	279 23	0.88 .07	0.75 .08	1.00 .05	No endocrine therapy for 1 year
2	215 4 ns	231 6 ns	0.94 .02 ↑ .05	52 3 ↓ .001	370 17 ↑ .001	419 14 ↑ .001	0.14 .01 ↓ .001	0.13 .01 ↓ .001	0.24 .02 ↓ .001	50 mg./day 17-methyl nortestosterone for 10 days
3	204 2 ns	243 4 ns	0.84 .01 ↓ .01	209 8 ↑ .001	285 6 ↓ .001	314 6 ↓ .001	0.74 .04 ↑ .001	0.67 .04 ↑ .001	1.03 .04 ↑ .001	Stop above Rx
L. R. 3	213 5	290 8	0.73 .01	336 5	196 7	228 8	1.72 .08	1.48 .06	1.57 .04	No medication for 3 months
4	131 4 ↓ .001	187 3 ↓ .001	0.71 .02 ns	47 2 ↓ .001	215 1 ↑ .02	249 4 ↑ .05	0.22 .01 ↓ .001	0.19 .01 ↓ .001	0.36 .03 ↓ .001	20 mg./day 17-methyl nortestosterone for 10 days
5	190 3 ↑ .001	257 3 ↑ .001	0.74 .01 ns	303 8 ↑ .001	194 6 ↓ .02	218 8 ↓ .01	1.57 .08 ↑ .001	1.40 .06 ↑ .001	1.60 .06 ↑ .001	Stop above Rx
M. R. 4	300 6	336 7	0.89 .02	330 6	361 7	390 7	0.92 .03	0.85 .02	1.10 .03	No medication for 3 months
5	311 1 ns	347 9 ns	0.90 .02 ns	189 19 ↓ .001	451 10 ↑ .001	485 19 ↑ .01	0.42 .05 ↓ .001	0.39 .06 ↓ .001	0.61 .06 ↓ .001	50 mg./day 17-methyl nortestosterone for 8 days
6	296 6 ↓ .05	325 7 ns	0.91 .03 ns	319 15 ↑ .01	379 4 ↓ .001	397 7 ↓ .01	0.84 .04 ↑ .001	0.80 .03 ↑ .001	1.08 .05 ↑ .001	Stop above Rx
G. M. 3	200 3	256 5	0.78 .01	209 2	220 2	309 10	0.95 .02	0.67 .02	1.05 .02	No medication for 2 months
4	242 12 ↑ .01	298 21 ns	0.82 .03 ns	65 4 ↓ .001	359 27 ↑ .01	392 27 ↑ .05	0.19 .02 ↓ .001	0.17 .02 ↓ .001	0.27 .03 ↓ .001	200 mg./day 17-methyl nortestosterone for 16 days
5	202 6 ↓ .02	245 7 ↓ .05	0.82 .02 ns	197 8 ↑ .001	236 21 ↓ .01	309 25 ns	0.86 .09 ↑ .001	0.65 .05 ↑ .001	0.98 .05 ↑ .001	Stop above Rx
L. R. 5	190 3	257 3	0.74 .01	303 8	194 6	218 8	1.57 .08	1.40 .06	1.60 .06	No medication for 3 months
6	161 1 ↓ .001	193 3 ↓ .001	0.84 .01 ↑ .001	122 4 ↓ .001	257 10 ↑ .001	277 8 ↑ .001	0.48 .01 ↓ .001	0.44 .01 ↓ .001	0.75 .02 ↓ .001	20 mg./day 17-ethyl nortestosterone for 14 days
M. R. 6	296 6	325 7	0.91 .03	319 15	379 4	397 7	0.84 .04	0.80 .03	1.08 .05	No medication for 3 months
7	306 19 ns	340 6 ns	0.90 .04 ns	161 31 ↓ .01	572 46 ↑ .01	616 56 ↑ .01	0.29 .07 ↓ .001	0.27 .07 ↓ .001	0.54 .13 ↓ .01	50 mg./day 17-ethyl nortestosterone for 17 days

NOTE: Lipids and lipoproteins in mg. per cent ± S.E. Significance determined by t test; arrow indicates direction of change.

times in twenty-nine such experiments. Lower density lipoprotein concentrations changed in the opposite direction twelve times, underwent no significant change nine times, and changed in the same direction as the high density lipoproteins five times. In other words, 81 per cent of the time the lower density lipoproteins either varied inversely or not at all with respect to the high density lipoproteins. Keeping this in mind, again it is not surprising to note in Table VIII that, in relation to estrogen therapy, serum cholesterol and phospholipid concentrations underwent either no change or varied in the same direction as the high density lipoproteins 88 per cent $\left(\frac{16+7}{26} \times 100 \text{ for cholesterol}\right)$ to 100 per cent $\left(\frac{18+8}{26} \times 100 \text{ for phospholipid}\right)$ of the time.

In other words, the response of serum cholesterol and phospholipid levels to gonadal steroid administration may be deduced or predicted if the behavior of the major high and lower density lipoprotein fractions is known. When these two major fractions tend to vary inversely, as is usually the case when gonadal steroids are administered, little change in the chemically determined levels of serum cholesterol or phospholipid is noted. If no significant change occurs in the lower density lipoprotein concentration, while the high density lipoproteins increase or decrease when estrogens or androgens are administered, then the serum cholesterol and phospholipid levels may be confidently predicted to change in the same direction as that of the high density lipoproteins.

The instances in which the lower density lipoproteins changed in the same direction as the high density lipoproteins deserve some mention. This is noted in Table VIII to have occurred seven times with androgens and five times with estrogens. This response usually followed the discontinuance of relatively weak androgens or relatively low doses of androgens (A. B.$_6$, J. P.$_3$, G. S.$_3$, W. P.$_{2,4}$ and G. T.$_4$), although there was one exception (A. B.$_5$). This response was also noted in two sisters of premenstrual age (S. T.$_{1,2}$ and E. T.$_{1,2}$) when estrone sulfate and equilin sulfate were administered.

It is not evident from the data what determines the direction of change in lower density lipoproteins when change occurs following administration or withdrawal of gonadal steroids.

TABLE V

EFFECTS OF TESTOSTERONE PROPIONATE AND SUBLINGUAL TESTOSTERONE ON SERUM LIPIDS AND LIPOPROTEINS

Subject	Cholesterol			Phospholipid			C/P Ratio			$-S_{1.21}$ 0–12 (alpha)			$-S_{1.21}$ 25–40 (beta)			$-S_{1.21}$ 25–70			$\frac{-S_{1.21}\ 0\text{–}12}{S_{1.21}\ 25\text{–}40}$			$\frac{-S_{1.21}\ 0\text{–}12}{25\text{–}70}$			$\frac{-S_{1.21}\ 0\text{–}12}{\text{Total Cholesterol}}$			Therapy
W. P. 1	257	2		332	14		0.78	.04		73	14					467	20		0.21	.04		0.17	.03		0.28	.04		No Rx for 23 weeks
2	227	19	ns	289	14	ns	0.79	.07	ns	74	1	ns	342	16		315	9	ns	0.23	.01	ns	0.20	.01	ns	0.33	.03	ns	25 mg. testosterone propionate in oil intramuscularly every 2 days for 15 weeks
3	297	7	↑ .02	310	8	ns	0.97	.03	ns	100	9	↑ .05	396	13	↑ .01	472	4	↑ .01	0.25	.03	ns	0.21	.02	ns	0.33	.03	ns	Stop above Rx; no Rx for 12 weeks
4	256	10	↓ .01	249	7	↓ .01	1.03	.04	ns	72	5	↓ .05	445	19	ns	494	15	ns	0.16	.01	↓ .02	0.15	.01	↓ .02	0.28	.03	ns	50 mg. testosterone propionate in oil intramuscularly every 2 days for 6 weeks
5	351	9	↑ .01	353	3	↑ .001	0.99	.02	ns	99	6	↑ .01	532	13	↑ .01	607	15	↑ .001	0.16	.02	ns	0.14	.02	ns	0.28	.01	ns	Stop above Rx
G. T. 3	147	10		242	8		0.61	.04		132	8		135	7		173	7		0.98	.01		0.76	.02		0.90	.08		No Rx for 6 weeks
4	127	6	ns	217	4	ns	0.59	.04	ns	123	1	ns	129	4	ns	161	2	ns	0.95	.03	ns	0.76	.01	ns	0.97	.05	ns	100 mg. testosterone propoinate in oil intramuscularly biweekly for 11 weeks
5	166	4	↑ .02	226	15	ns	0.74	.04	↑ .05	143	6	↑ .05	163	12	↑ .05	188	13	ns	0.88	.06	ns	0.76	.04	ns	0.86	.02	ns	Stop above Rx
L. S. 1	168	13		220	9		0.76	.05		178	2		191	7		214	8		0.93	.05		0.84	.04		1.08	.10		No Rx
2	179	7	ns	263	4	↑ .01	0.68	.02	ns	168	9	ns	230	10	↑ .02	260	11	↑ .01	0.74	.05	↓ .02	0.65	.05	↓ .02	0.94	.03	ns	Sublingual testosterone 48–96 mg./day for 16 weeks
3	170	22	ns	247	12	ns	0.68	.06	ns	213	13	↑ .02	189	19	ns	205	9	↓ .01	1.15	.05	↑ .01	1.06	.09	↑ .01	1.27	.10	↑ .02	Stop above Rx

NOTE: Lipids and lipoproteins in mg. per cent ± S.E. Significance determined by t test; arrow indicates direction of change.

TABLE VI

EFFECTS OF ESTROGENS ON SERUM LIPIDS AND LIPOPROTEINS

Subject	Cholesterol	Phospholipid	C/P Ratio	$-S_{1.21}$ 0–12 (alpha)	$-S_{1.21}$ 25–40 (beta)	$-S_{1.21}$ 25–70	$-S_{1.21}\frac{0-12}{25-40}$	$-S_{1.21}\frac{0-12}{25-70}$	$\frac{-S_{1.21}\ 0-12}{\text{Total Cholesterol}}$	Therapy
M. C. 1	207 5	293 10	0.71 .01	215 5	226 10	289 11	0.95 .02	0.75 .01	1.03 .01	No Rx
2	209 6 ns	343 12 ↑ .05	0.61 .02 ↓ .01	365 12 ↑ .01	174 11 ↓ .01	241 13 ↓ .01	2.13 .17 ↑ .001	1.53 .11 ↑ .001	1.75 .09 ↑ .001	Stilbestrol 2 mg./day for 77 days
3	149 8 ↓ .001	254 13 ↓ .01	0.59 .03 ns	226 8 ↓ .001	197 6 ns	238 12 ns	1.15 .06 ↓ .01	0.95 .02 ↓ .01	1.53 .11 ns	Stop above Rx
Z. D. 1	229 8	358 10	0.64 .03	378 11	223 15	238 17	1.71 .09	1.60 .10	1.66 .08	Stilbestrol 5 mg./day for 77 days
2	219 7 ns	294 16 ↓ .01	0.75 .06 ↑ .01	235 25 ↓ .01	318 16 ↑ .01	335 14 ↑ .01	0.74 .09 ↓ .001	0.70 .07 ↓ .001	1.07 .11 ↓ .01	Stop above Rx
M. P. 1	231 12	368 20	0.63 .02	319 12	284 24	323 18	1.08 .17	0.94 .10	1.40 .10	Stilbestrol 3 mg./day for 68 days
2	216 11 ns	317 4 ↓ .05	0.68 .03 ns	248 6 ↓ .001	278 31 ns	335 21 ns	0.93 .08 ns	0.75 .04 ns	1.17 .08 ns	Stop above Rx
J. S. 1	165 25	288 9	0.57 .07	231 16	134 10	177 11	1.72 .06	1.30 .01	1.44 .15	Stilbestrol 3 mg./day for 86 days
2	163 15 ns	262 11 ns	0.62 .05 ns	137 9 ↓ .01	170 6 ↑ .02	236 12 ↑ .01	0.81 .05 ↓ .001	0.59 .05 ↓ .001	0.85 .03 ↓ .01	Stop above Rx
A. T. 1	221 13	289 25	0.77 .12	223 9	229 18	261 16	0.99 .12	0.86 .08	1.02 .10	No Rx
2	190 7 ns	289 14 ns	0.66 .01 ns	306 17 ↑ .01	167 5 ↓ .02	217 11 ↓ .05	1.87 .16 ↑ .01	1.43 .10 ↑ .01	1.61 .05 ↑ .001	Premarin 10 mg./day for 35 days then stilbestrol 3 mg./day for 45 days
3	159 — ns	225 — ns	0.71 — ns	115 — ↓ .01	303 — ↑ .01	403 — ↑ .01	0.38 — ↓ .01	0.28 — ↓ .01	0.72 — ↓ .001	Stop above Rx
G. S. 1	188 7	318 9	0.59 .01	333 9	184 8	243 10	1.82 .07	1.38 .04	1.79 .08	Premarin 10–25 mg./day for 100 days
2	209 9 ns	294 14 ns	0.71 .01 ↑ .001	285 11 ↓ .01	241 11 ↑ .01	241 11 ns	1.18 .01 ↓ .001	1.18 .01 ↓ .01	1.37 .11 ↓ .02	Stop above Rx
W. W. 1	122 7	212 8	0.58 .02	219 10	180 8	215 2	1.23 .05	1.04 .06	1.81 .08	No Rx
2	145 10 ns	218 6 ns	0.66 .04 ns	222 11 ns	176 10 ns	211 15 ns	1.26 .05 ns	1.03 .02 ns	1.54 .06 ↓ .05	Estrone sulfate 5 mg./day for 25 days, then premarin 5 mg./day for 24 days
3	148 6 ns	226 2 ns	0.65 .03 ns	196 22 ns	173 6 ns	201 11 ns	1.13 .10 ns	0.97 .06 ns	1.34 .21 ns	Stop above Rx
S. T. 1	126 5	229 22	0.56 .03	178 5	180 3	202 7	0.99 .07	0.88 .06	1.42 .17	No Rx
2	163 1 ↑ .01	264 39 ns	0.63 .09 ns	257 11 ↑ .01	218 20 ↑ .01	298 28 ↑ .01	1.19 .06 ns	0.87 .05 ns	1.57 .07 ns	Estrone sulfate 5 mg./day for 100 days
3	135 3 ↓ .01	198 10 ns	0.68 .04 ns	146 2 ↓ .01	167 6 ↓ .05	197 7 ↓ .05	0.88 .02 ↓ .01	0.74 .03 ↓ .05	1.09 .02 ↓ .01	Stop above Rx
M. C. 3	149 8	254 13	0.59 .03	226 8	197 6	238 12	1.15 .06	0.95 .02	1.53 .11	No Rx for 96 days
4	179 8 ↑ .02	317 10 ↑ .01	0.56 .01 ns	341 11 ↑ .001	171 8 ↓ .02	217 13 ns	2.01 .10 ↑ .001	1.59 .10 ↑ .001	1.95 .12 ↑ .05	Equilin sulfate 2.5 mg./day for 78 days
5	187 16 ns	295 16 ns	0.63 .03 ns	272 16 ↓ .001	255 6 ↑ .001	297 20 ↑ .02	1.07 .05 ↓ .001	0.92 .03 ↓ .001	1.47 .06 ↓ .01	Stop above Rx
M. H. 1	155 4	253 4	0.61 .01	243 8	199 14	234 12	1.23 .05	1.04 .01	1.56 .06	Equilin sulfate 10 mg./day for 43 days
2	163 7 ns	251 6 ns	0.65 .04 ns	211 7 ↓ .05	228 8 ns	269 11 ns	0.93 .01 ↓ .01	0.78 .01 ↓ .01	1.30 .06 ↓ .02	Stop above Rx
M. M. 2	243 7	293 5	0.83 .02	313 13	239 9	282 8	1.32 .07	1.12 .06	1.30 .07	No Rx for 124 days
3	219 6 ↓ .05	319 5 ↑ .01	0.69 .01 ↑ .001	361 16 ↑ .05	210 7 ↓ .05	251 9 ↓ .05	1.74 .10 ↑ .01	1.45 .10 ↑ .02	1.65 .10 ↑ .02	Equilin sulfate 2.5 mg./day for 78 days
4	229 2 ns	295 7 ↓ .05	0.78 .01 ↓ .001	360 6 ns	263 5 ↑ .001	288 4 ↑ .01	1.37 .04 ↓ .01	1.25 .04 ns	1.57 .04 ns	Stop above Rx
M. R. 1	227 7	321 8	0.71 .03	315 12	269 6	291 6	1.18 .07	1.09 .05	1.39 .07	No Rx for 162 days
2	257 16 ns	383 11 ↑ .001	0.67 .04 ns	412 13 ↑ .001	261 19 ns	297 18 ns	1.59 .08 ↑ .001	1.39 .06 ↑ .01	1.63 .15 ns	Equilin sulfate 2.5 mg./day for 56 days
3	240 7 ns	323 14 ↓ .01	0.75 .03 ns	314 8 ↓ .001	312 10 ↑ .05	335 9 ns	1.02 .06 ↓ .001	0.94 .04 ↓ .001	1.31 .06 ns	Stop above Rx
E. T. 1	113 7	195 25	0.59 .11	195 25	148 19	179 30	1.36 .34	1.15 .33	1.75 .32	No Rx
2	174 7 ↑ .01	290 8 ↑ .02	0.60 .01 ns	267 2 ↑ .05	210 13 ↑ .05	242 16 ns	1.29 .09 ns	1.12 .09 ns	1.54 .06 ns	Equilin sulfate 2.5 mg./day for 106 days
3	161 3 ns	222 7 ↓ .001	0.73 .02 ↑ .01	212 10 ↓ .01	168 9 ↓ .05	208 15 ns	1.27 .03 ns	1.03 .05 ns	1.32 .08 ns	Stop above Rx
W. W. 5	120 7	201 6	0.60 .04	158 5	138 2	174 2	1.14 .04	0.91 .04	1.33 .08	No Rx for 125 days
6	139 8 ns	259 4 ↑ .001	0.54 .02 ns	284 4 ↑ .001	162 5 ↑ .01	206 5 ↑ .01	1.75 .07 ↑ .001	1.38 .04 ↑ .001	2.03 .10 ↑ .001	Equilin sulfate 5 mg./day for 49 days
7	147 17 ns	242 3 ↓ .05	0.60 .07 ns	227 10 ↓ .01	173 3 ns	217 31 ns	1.31 .04 ↓ .01	1.06 .12 ↓ .01	1.58 .07 ↓ .02	Stop above Rx
Z. D. 3	259 12	308 3	0.84 .05	277 6	251 3	272 2	1.11 .03	1.02 .03	1.08 .07	No Rx for 55 days
4	226 6 ↓ .05	357 3 ↑ .001	0.63 .01 ↓ .01	368 15 ↑ .001	215 10 ↓ .01	255 9 ↓ .05	1.74 .15 ↑ .01	1.47 .11 ↑ .01	1.71 .12 ↑ .01	Non-ketonic estrogens 5 mg./day for 77 days
5	225 9 ns	292 5 ↓ .001	0.78 .03 ↑ .01	249 8 ↓ .001	268 20 ↑ .01	293 18 ↑ .05	0.97 .09 ↓ .001	0.87 .07 ↓ .01	1.11 .05 ↓ .001	Stop above Rx
M. M. 1	201 10	338 3	0.59 .02	365 7	213 10	263 9	1.72 .08	1.39 .06	1.83 .06	Non-ketonic estrogens 3 mg./day for 98 days
2	243 7 ↑ .01	293 5 ↓ .001	0.83 .02 ↑ .001	313 13 ↓ .01	239 9 ns	282 8 ns	1.32 .07 ↓ .01	1.12 .06 ↓ .01	1.30 .07 ↓ .001	Stop above Rx
W. W. 8	151 11	253 4	0.61 .04	254 7	194 5	231 6	1.31 .04	1.10 .02	1.68 .13	Non-ketonic estrogens 3 mg./day for 77 days
9	135 2 ns	223 10 ↓ .05	0.61 .02 ns	196 7 ↓ .01	184 7 ns	207 10 ns	1.07 .08 ↑ .05	0.95 .08 ns	1.45 .08 ns	Stop above Rx
W. P. 1	351 9	353 3	0.99 .02	99 6	531 13	607 15	0.19 .01	0.16 .01	0.28 .01	No Rx for 81 days
2	319 4 ↓ .02	317 4 ↓ .001	1.01 .02 ns	144 4 ↑ .001	528 7 ns	592 4 ns	0.27 .01 ↑ .001	0.24 .01 ↑ .001	0.45 .01 ↑ .001	Estradiol benzoate (oil) 5 mg. intramuscularly every 2 days for 24 days
3	355 7 ↑ .01	326 5 ↑ .02	1.06 .02 ns	118 5 ↓ .01	515 28 ns	601 37 ns	0.23 .01 ↓ .01	0.20 .01 ↓ .02	0.34 .02 ↓ .001	Stop above Rx

NOTE: Lipids and lipoproteins in mg. per cent ± S.E. Significance determined by t test; arrow indicates direction of change.

It is likely that the qualitative as well as the quantitative responses of the serum lipoproteins to the administration of gonadal steroids are influenced not only by the amount of steroid administered but by the endocrinologic and/or metabolic status of the individual as well. Dietary habits may play a role in determining the lipoprotein response to gonadal steroid administration. With the exception of a few subjects studied under metabolic balance conditions, no dietary restrictions were imposed during the course of this study.

No significant changes in low density lipoproteins were noted in the absence of significant changes in high density lipoproteins.

The ease with which the concentration of high density lipoproteins may be significantly and profoundly influenced by gonadal steroid administration suggests that fewer variables are involved in the homeostatic mechanism which determines the level of these lipoproteins in the blood at any given time, in contrast to the lower density $-S_{1.21}$ 25–40 and 25–70, etc. lipoproteins, the levels of which at any given moment are undoubtedly conditioned by caloric intake, nature and amount of dietary fat, serum lipoprotein lipase activity and probably other metabolic factors and phenomena as yet unrecognized. It is of interest to note that in a mammalian species such as the dog, in which the serum lipoproteins are mainly of the high density $-S_{1.21}$ 0–12 type, the total serum lipoprotein concentration as well as serum cholesterol and phospholipid levels may be markedly reduced by the administration of androgen [*64*].

The combined administration of gonadal steroids such as methyl testosterone 10 mg./day with stilbestrol 5 mg./day, or methyl testosterone 25 mg./day with premarin 20 mg./day, was associated with responses characteristic of androgen administration, i.e., a reduction in high density lipoproteins. (Fig. 2.)

TABLE VII

RESPONSE OF LOWER DENSITY $-S_{1.21}$ 25–40 LIPOPROTEINS IN RELATION TO THE DIRECTION OF CHANGE INDUCED IN HIGH DENSITY $-S_{1.21}$ 0–12 LIPOPROTEINS BY GONADAL STEROID THERAPY

Steroid	Opposite Direction	No Significant Change	Same Direction	Totals
Androgen...	29	16	7	52*
Estrogen....	12	9	5	26*

* Only statistically significant changes in high density lipoproteins were utilized for this compilation, hence the total for androgen is 52 (of 55), and for estrogen is 26 (of 29).

TABLE VIII

RESPONSE OF SERUM CHOLESTEROL AND PHOSPHOLIPIDS IN RELATION TO DIRECTION OF CHANGE INDUCED IN HIGH DENSITY $-S_{1.21}$ 0–12 LIPOPROTEINS BY GONADAL STEROID THERAPY

Steroid	Opposite Direction	No Significant Change	Same Direction	Totals
Cholesterol				
Androgen...	8*	22	22	52†
Estrogen....	3	16	7	26†
Phospholipid				
Androgen...	5*	21	26	52†
Estrogen....	0	8	18	26†

* This figure includes four responses in two subjects (G. B. No. 40 and G. M. No. 484) in both of whom hypercholesterolemia and hyperphospholipemia developed while they were receiving methyl testosterone.

† Only statistically significant changes in high density lipoproteins were utilized for this compilation, hence the total for androgen is 52 (of 55), and for estrogen is 26 (of 29).

The *in vitro* addition of water-soluble estrogen or androgen to serum effected no change in the serum lipid or lipoprotein patterns of the serums so treated.

The tendency for serum phospholipid levels to vary in the same direction as the high density lipoproteins during either androgen or estrogen therapy (Figs. 2, 3 and 4) is undoubtedly due in important measure to the fact that high density lipoproteins have lower cholesterol/phospholipid ratios than the lower density lipoproteins [*45*]. The tendency of phospholipid levels to vary with the high density lipoproteins seemed slightly greater when estrogens were employed. (Table VIII.)

Of interest is the observation that in two subjects during the course of therapy with methyl testosterone (G. B. and G. M.) marked hypercholesterolemia developed. (Fig. 1.) Subject G. B. had become deeply jaundiced during a

previous course of methyl testosterone, with a lipoprotein pattern characteristic of biliary obstruction [*65*] i.e., marked reduction in high density lipoprotein concentration. During the course of this jaundice he was moderately hypercholesterolemic. As hypercholesterolemia again developed, methyl testosterone was continued and careful watch kept for evidence of jaundice, both clinically and by serum bilirubin measurement. Although all ultracentrifugal evidence of high density lipoproteins disappeared, jaundice did not recur and methyl testosterone was eventually discontinued. The mechanism responsible for the development of hypercholesterolemia in these two subjects is not known, nor is the rare appearance of jaundice in subjects taking methyl testosterone understood. The latter has been described previously and attributed to stasis of bile in the bile canaliculi [*66*].

The urinary 17-ketosteroid excretion was not necessarily related to the magnitude of change induced in the lipoprotein spectrum by androgens. (Figs. 2 and 3.) Thus administration of a highly effective agent for altering lipoproteins, methyl testosterone, was not followed by increased 17-ketosteroid excretion, while sublingual testosterone administration, was regularly followed by large increments in urinary 17-ketosteroids, was largely ineffective in altering lipoproteins. (Fig. 2.) Likewise, administration of nortestosterone, a relatively weak oral androgen when compared with 17-methyl or 17-ethyl nortestosterone [*67*], was followed by increased 17-ketosteroid excretion, while the C-17 substituted derivatives were not. It is evident therefore that androgen-induced changes in the lipoproteins of human serum are not dependent on the presence of intermediates of steroid metabolism which are excreted as 17-ketosteroids.

In subjects with primary gonadal insufficiency, changes in urinary gonadotropin excretion following gonadal steroid administration were slower in appearing than were the serum lipoprotein changes. (Fig. 4.) Using androgens, it was possible to induce significant alteration of the serum lipoprotein spectrum without detectable change in urinary gonadotropin excretion. (Fig. 3.) In the female, only the vaginal smear was a more sensitive indicator of estrogen administration.

The response of high density lipoproteins characteristic of gonadal steroid administration can be elicited in a variety of subjects of either sex, ranging from apparently healthy subjects to individuals with various endocrinopathies or diseases. In adrenal hyperplasia the administration of cortisone has been associated with increases in high density lipoprotein concentrations [*68*], a response characteristic of estrogen in other subjects. In severe chronic biliary obstruction estrogens appear relatively ineffective in increasing high density $-S_{1.21}$ 0–12 lipoprotein levels.

Since the metabolic and androgenic or estrogenic properties of gonadal steroids have not yet been proved to be completely separable, changes in the lipoprotein spectrum induced by these agents provide a ready index of such properties.

In contrast to the protein anabolic effect of testosterone, which may show a "wearing off" phenomenon after prolonged administration, neither prolonged androgen nor estrogen administration was associated with any tendency for the serum lipoprotein changes to subside or otherwise "escape." (Figs. 1 and 4.) Furthermore, in four subjects undergoing metabolic balance study in whom positive nitrogen balance had been induced by androgen administration, the return of nitrogen balance to control levels following cessation of androgen preceded the return of the lipoprotein spectrum to the control state, often by a week or more. The importance of this observation in metabolic or other type studies where control states must be re-established after steroid therapy is self-evident.

The observations reported here indicate that any study of the serum lipoproteins in health or disease must include the full spectrum of lipoproteins if adequate evaluation of their importance is to be made. If the comparative freedom from coronary atherosclerosis which women enjoy is related to a particular serum lipoprotein spectrum which is engendered by a relative abundance of estrogenic steroids, then the focus of attention must be shifted from the S_f lipoprotein classes exclusively, and a more comprehensive perspective employed. Recently reported studies [*69*] of the "predictive value" of certain S_f lipoprotein classes in categorizing individuals likely to manifest coronary artery disease illustrate the need for this more comprehensive approach, inasmuch as the considerable effort devoted to these studies has not resulted in agreement among the investigators, nor has it produced clear-cut results. The sensitivity of high density lipoprotein concentrations to change induced by gonadal steroid administration indi-

cates that, if one or more classes of lipoproteins are etiologically related to coronary atherosclerosis, then such class or classes of lipoproteins must be considered within the framework of the total lipoprotein spectrum. Here a relationship such as the ratio of high density to lower density lipoprotein concentrations may be of great significance [*70*], and it may be that women tend to have less coronary atherosclerosis than men because of higher values for this particular ratio.

SUMMARY AND CONCLUSIONS

Data representative of a four-year study of the influence of gonadal steroids on serum lipids and lipoproteins in more than sixty human subjects are presented. Estrogens promptly and consistently increase high density $-S_{1.21}$ 0–12 (alpha) lipoprotein concentrations, while androgens promptly lower them. The concentration of lower density $-S_{1.21}$ 25–40 (beta) or $-S_{1.21}$ 25–70 lipoproteins characteristically either does not change, or changes in a direction opposite to that of the high density lipoproteins, when gonadal steroids are administered. The response of the chemically determined serum cholesterol and phospholipid concentrations may be predicted on the basis of the lipoprotein changes induced by gonadal steroids in individual subjects. When the concentrations of high density and lower density lipoprotein fractions change in opposite directions, serum cholesterol and phospholipid levels show little or no change. When lower density lipoprotein concentrations fail to change following gonadal steroid exhibition, then serum cholesterol and phospholipid levels change in the same direction as that of the high density lipoproteins. Serum phospholipids tend to vary with the high density lipoprotein concentrations more than does the serum cholesterol, presumably because of the relatively high phospholipid/cholesterol ratio of the $-S_{1.21}$ 0–12 lipoproteins.

Changes induced in the serum lipoproteins by gonadal steroids tend to persist for one or more weeks after therapy is discontinued and after nitrogen balance has returned to the control state. No "wearing off" phenomena have been observed. The responses of the lipoproteins do not appear to be conditioned by urinary gonadotropin or 17-ketosteroid excretion, and may be elicited in hypothyroidism and panhypopituitarism.

The changes induced in the serum lipoproteins of human subjects by gonadal steroid administration suggest that the relative immunity of women to coronary atherosclerosis may be attributable either to relatively large concentrations of high density lipoproteins or to relatively high values for high density/lower density lipoprotein ratios. The data also indicate the necessity of study of the entire serum lipoprotein spectrum if the physiologic or etiologic importance of these lipoproteins in health and disease is to be fully evaluated.

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APPENDIX

Case histories of patients whose data are presented.

A. B. (No. 39). A thirty-two year old man, 69 kg., 183 cm. *Hypogonadotropic eunuchoidism.* X-ray sella normal. Cardiovascular system normal. Steroids studied: methyl testosterone supplemented by testosterone alone (two courses), methyl testosterone combined with premarin, testosterone (sublingual) and nortestosterone.

G. B. (No. 40). A thirty-one year old man, 85 kg., 173 cm. *Hypogonadotropic eunuchoidism.* X-ray sella normal. Cardiovascular system normal. During second course of methyl testosterone therapy painless jaundice developed with laboratory evidence of

obstruction; later cholecystogram was normal. Subsequent course of methyl testosterone was not accompanied by jaundice. Steroids studied: methyl testosterone (three courses) and premarin.

H. B. (No. 213). A twenty year old man, 93 kg., 194 cm. *Hypergonadotropic eunuchoidism.* Large breasts amputated. Cardiovascular system normal. Steroids studied: methyl testosterone.

S. B. (No. 4). An eighty-three year old woman, 55 kg., 150 cm. *Osteoporosis.* Severe vertebral body wedging and kyphosis. Blood chemistry and bone marrow normal. Natural menopause. Chronic pyelonephritis controlled by medication. Recurrent partial intestinal obstruction from adhesions. Cardiovascular system normal. Steroids studied: methyl testosterone (two courses), methyl testosterone combined with premarin, methyl testosterone combined with stilbestrol, nortestosterone and allopregnanolone.

M. C. (No. 316). A seventeen year old girl, 49 kg., 136 cm. *Gonadal agenesis.* Typical Turner's syndrome. Gonadotropins elevated. Cardiovascular system normal. Blood pressure, 120/74 mm. Hg. Steroids studied: stilbestrol, equilin sulfate.

Z. D. (No. 267). A seventy-eight year old woman, 70 kg., 140 cm. *Osteoporosis.* Wedged spinal vertebrae. Blood chemistry and bone marrow normal. Natural menopause. Exertional dyspnea, electrocardiogram showed extrasystoles. Blood pressure, 172/100 mm. Hg. Steroids studied: methyl testosterone, stilbestrol, non-ketonic estrogen mixture, allopregnanolone.

M. H. (No. 18). A forty-seven year old woman, 74 kg., 158 cm. *Osteoporosis.* Collapsed dorsal vertebra discovered four years previously when simple hysterectomy performed for fibroids. Pelvic x-ray therapy postoperatively. Gonadotropins elevated. Cardiovascular system normal. Steroids studied: testosterone propionate (oil) intramuscular, 17-methyl nortestosterone, estrone sulfate, equilin sulfate, progesterone (sublingual), allopregnanolone.

P. H. (No. 571). A forty-one year old man, 89 kg., 188 cm. *No disease.* Steroid studied: methyl testosterone.

G. M. (No. 484). A twenty-four year old man, 75 kg., 168 cm. *Myotonic dystrophy.* Slight muscular weakness. Testes small, gonadotropins elevated. Cardiovascular system normal. Steroids studied: methyl testosterone, 17-methyl nortestosterone.

J. M. (No. 110). A twenty-one year old man, 63 kg., 171 cm. *True hermaphroditism.* Right ovary, uterus and breasts removed. Left scrotal ovotestis biopsied. Gonadotropins elevated. Epidermal chromatin pattern female. Previous androgen therapy masculinized hair pattern, voice and libido. Cardiovascular system normal. Steroid studied: methyl testosterone.

M. M. (No. 130). A twenty-eight year old woman, 53 kg., 159 cm. *Pseudohermaphroditism.* Female external genitalia. One gonad excised from groin in infancy. Second gonad descended when patient was twenty-six years old and proved to be atrophic testis. Gonadotropins elevated. Cardiovascular system normal. Steroids studied: equilin sulfate non-ketonic estrogen mixture.

R. M. (No. 486). A fifty-seven year old man, 69 kg., 167 cm. *Myotonic dystrophy.* Father of G. M. (No. 484). Severe muscular weakness. Testes small, gonadotropins elevated. Slight congestive cardiac failure; generalized arteriosclerosis; electrocardiogram, normal; blood pressure 138/74 mm. Hg. Steroids studied: methyl testosterone. Died months later with coronary thrombosis and pneumonitis.

J. P. (No. 205). A forty-seven year old man, 90 kg., 178 cm. *Hypergonadotropic eunuchoidism.* Osteoporosis, collapsed spinal vertebrae. Occasional angina; electrocardiogram, normal; blood pressure, 88/58 mm. Hg. Steroids studied: methyl testosterone (two courses), testosterone (sublingual).

M. P. (No. 46). A seventy year old woman, 85 kg., 165 cm. *Osteoporosis.* Collapsed spinal vertebrae. Blood chemistry and bone marrow normal. Natural menopause. Non-toxic goiter. Exertional dyspnea; angina; electrocardiogram, normal; blood pressure, 150/88 mm. Hg. Steroids studied: testosterone isobutyrate (aqueous) intramuscular, testosterone propionate (aqueous) intramuscular, methyl testosterone, nortestosterone, stilbestrol.

W. P. (No. 119). A fifty-six year old man, 90 kg., 178 cm. *Panhypopituitarism.* Onset twenty years previously with transient diabetes insipidus, progressive weakness, loss of libido, myxedema. Given two courses of deep x-ray therapy to pituitary region, but visual impairment followed. Chromophobe adenoma removed from hypophysis ten months before study. After withdrawal of cortisone and thyroid medications no hypoglycemia developed, but adrenal and thyroid function markedly impaired. Gonadotropins absent. Cardiovascular system normal. Blood pressure, 130/80 mm. Hg. Steroids studied: testosterone propionate (oil) intramuscular (2 courses), estradiol benzoate (oil) intramuscular.

L. R. (No. 502). A sixty-nine year old woman, 53 kg., 152 cm. *Osteoporosis.* Collapsed spinal vertebrae. Blood chemistry and bone marrow normal. Natural menopause. Carcinoma of cervix treated by radium three years previously, recent pelvic examination negative. Cardiovascular system normal. Steroids

studied: nortestosterone, methyl nortestosterone, ethyl nortestosterone.

M. R. (No. 236). A forty-four year old woman, 111 kg., 166 cm. *Surgical castration.* Cardiovascular system normal. Steroids studied: methyl testosterone, methyl nortestosterone, ethyl nortestosterone, estrone sulfate, equilin sulfate.

G. S. (No. 30). A seventy-nine year old woman, 42 kg., 151 cm. *Osteoporosis.* Severely wedged spinal vertebrae. Blood chemistry and bone marrow normal. Natural menopause. Hematemesis from peptic ulcer. Cardiovascular system normal. Steroids studied: methyl testosterone, nortestosterone (two courses), premarin.

J. S. (No. 228). A fifty-two year old woman, 85 kg., 152 cm. *Myxedema, primary.* Gradual onset twenty years previously with marked exertional dyspnea recently. Low thyroidal I-131 uptake, unresponsive to TSH. Natural menopause nine years previously. Gonadotropins elevated. Moderate cardiac enlargement. Ascites and dependent edema. Electrocardiogram showed rate 75/minute, LVH and primary T-wave changes. Blood pressure 210/110 to 150/90 mm. Hg. Steroid studied: stilbestrol. Although subsequent thyroid therapy relieved all symptoms, cardiac enlargement and moderate hypertension persisted.

L. S. (No. 324). A twenty-eight year old man, 61 kg., 185 cm. *Hypergonadotropic eunuchoidism.* Subtotal thyroidectomy nine years previously. Decreased masculine body hair pattern. No gynecomastia. Bilateral cryptorchidism with inguinal hernias. Cardiovascular system normal. Steroid studied: testosterone (sublingual).

A. T. (No. 298). A twenty-eight year old man, 46 kg., 181 cm. *Third degree burns.* Burned twelve months previously. Both legs atrophic, joints ankylosed. Renal calculus. Cardiovascular system normal. Steroids studied: premarin, stilbestrol.

E. T. (No. 412). A fourteen year old girl, 37 kg., 162 cm. *Delayed puberty.* Little subcutaneous tissue, long extremities, no pubic or axillary hair or breast development. Gonadotropins absent. X-ray sella normal. Cardiovascular system normal. Steroids studied: equilin sulfate.

G. T. (No. 443). A nineteen year old man, 80 kg., 190 cm. *Hypergonadotropic eunuchoidism.* Slight gynecomastia. Minimal active pulmonary tuberculosis. Cardiovascular system normal. Steroids studied: nortestosterone, testosterone propionate (oil) intramuscular.

S. T. (No. 335). A seven and a half year old girl, 18 kg., 115 cm. *Osteogenesis imperfecta.* Familial history. Blue sclerae. Only two fractures in past, but x-rays showed thin bones, blood chemistry normal. No abnormalities in genital or cardiovascular system. Steroids studied: estrone sulfate.

W. W. (No. 240). A twenty-two year old woman, 50 kg., 169 cm. *Endometriosis, bilateral ovarian resection.* Amenorrhea during intervening eighteen months. Gonadotropins elevated. Cardiovascular system normal. Steroids studied: estrone sulfate, premarin, equilin sulfate, non-ketonic estrogen mixture, progesterone (sublingual), progesterone (oil) intramuscular.

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Review

Right-sided Bacterial Endocarditis and Endarteritis*

A Clinical and Pathologic Study

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BACTERIAL endocarditis has a decided preference for structures of the left side of the heart, and it is this location of the infection that is most widely described and understood. In an attempt better to delineate the characteristics of right-sided bacterial endocarditis, and to review mechanisms important in the development and localization of bacterial endocarditis, the present study was undertaken.

As early as the mid-nineteenth century clinicians were aware that left-sided endocarditis occurs more frequently than does right-sided endocarditis. In 1852 Kirkes [*1*], in his classic treatise on emboli originating from the interior of the heart, pointed out that "the fibrinous deposits" from which emboli arise far more commonly affect the left-sided valves than those on the right. In subsequent years clinicians continued to be impressed by the infrequency of right-sided endocarditis [*2,3*].

Incidence. In the majority of large review series the incidence of isolated right-sided localization is given as about 5 per cent [*4,5*] of cases of bacterial endocarditis. It soon becomes apparent, however, that this figure can be markedly influenced by a number of factors. Thus it seems appropriate to discuss briefly the various factors important in the development of bacterial endocarditis, with particular reference to their influence on the localization of the vegetations.

The development of bacterial endocarditis is dependent on the summation of two factors: (1) the susceptibility of the endocardium to bacterial infection and (2) the infectiousness of the available organism. When the susceptibility of the endocardium is high, an organism of relatively low virulence is capable of producing bacterial endocarditis. In the presence of a normal heart, however, an intense and prolonged bacteremia of highly virulent organisms is usually required before bacterial endocarditis can develop.

Susceptibility of the Endocardium. The most important factors that enhance endocardial susceptibility to infection are (1) intracardiac pressure, (2) rheumatic valvulitis, (3) congenital malformation, and (4) host and endocrine factors. Although the first three factors may all exert their influence through the common denominator of stress or trauma, each will be considered separately.

Intracardiac Pressure: In view of the considerably greater frequency of left-sided than right-sided endocarditis, it is not surprising that early workers such as Bramwell [*6*] were quick to implicate the greater pressures and assumed resultant trauma to which the left-sided valves are subjected. Allen [*7*] combined several large series and determined the frequency of right-sided endocarditis to be 10.6 per cent. He also noted the pressure in the pulmonary artery to be 16.6 per cent of the aortic pressure, and expressed the view that these figures were sufficiently close to suggest a relationship between

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pressure and susceptibility to endocarditis. More recently Lepeschkin [*8*] presented additional evidence supporting the concept that pressure is important in determining the involvement of individual valves in rheumatic or bacterial endocarditis.

The basic mechanism whereby greater pressure in the left side of the heart enhances endocardial susceptibility to infection is probably through local stress and trauma. Both clinical and experimental evidence is available to emphasize the importance of these two factors in the development of bacterial endocarditis.

Clinically, it is known that points of increased trauma related to abnormal blood flow in congenital heart disease are often sites of predilection for bacterial vegetations [*9*]. Experimentally, Highman and Altland [*10*] have revealed the increased susceptibility of high altitude rats to experimental bacterial endocarditis, while a similar lowering of the threshold to bacterial infection has been noted in the hearts of dogs with experimental arteriovenous fistulas [*11,12*]. Factors found to be of importance in determining whether or not endocarditis will develop in an experimental animal are the degree and duration of increase in cardiovascular work load.

Rheumatic valvulitis: The frequency with which subacute bacterial endocarditis is superimposed upon pre-existing rheumatic valvular disease is well known [*13–16*]. Since rheumatic valvulitis far more commonly affects the left- than the right-sided valves [*5,17–19*], it undoubtedly enhances the frequency with which subacute bacterial endocarditis localizes in the left side of the heart.

On the other hand, when acute bacterial endocarditis is considered, the importance of rheumatic heart disease as a predisposing factor immediately diminishes. Thayer [*5*] found the incidence of pre-existing valvular disease to be 81.1 per cent in subacute bacterial endocarditis. In contrast, when organisms more prone to cause acute bacterial endocarditis were involved he found clinical or pathologic evidence of pre-existing valvular disease as follows: staphylococcal (aureus) endocarditis, 43 per cent; pneumococcal endocarditis, 37.5 per cent; and gonococcal endocarditis, 14.8 per cent.

As the importance of pre-existing rheumatic valvulitis diminishes, its influence on the left-sided localization of vegetations should likewise decrease and one would expect a greater frequency of right-sided endocarditis when the infection is acute. That this is true will be discussed in some detail later.

Congenital cardiac malformations: The presence of congenital cardiac malformations also increases the susceptibility of the heart to bacterial endocarditis [*20–21*]. Cardiac anomalies of particular importance in this regard are ventricular septal defect, congenital pulmonic stenosis, patent ductus arteriosus, tetralogy of Fallot and congenital bicuspid aortic valves [*21*]. In all but the last of these the bacterial infection tends to develop in the right side of the heart or pulmonary artery at sites where so-called jet lesions occur, or on congenitally deformed valves. Thus, certain congenital cardiac anomalies may actually increase the frequency of right-sided endocarditis.

Host and endocrine factors: Data collected by Lillehei and co-workers [*11,22*] have suggested to them that increased stress may result in alteration or imbalance of hormone secretion from the adrenal gland which somehow augments cardiac susceptibility to infection. Nevertheless, this and many other factors which make up the ability of the host to resist infection would not seem to affect the site at which vegetations localize, and will not be elaborated upon further in this discussion.

An additional situation that might influence the valvular distribution of endocarditis is the relative vascularity of the various valves. One would first have to assume that bacteria reach the valves through their own blood vessels via the coronary arteries, and there is very little evidence that this occurs [*15*]. Even if it did, the work of Wearn and Moritz [*23*] showed that when vascularization of the heart valves beyond the proximal third occurs, vessels are found twice as often in left-sided valves as in right-sided valves; also the tricuspid valve contains vessels more often than does the aortic valve. Obviously, this does not correspond to the relative frequency with which various valves are affected by bacterial endocarditis. Thus it seems unlikely that bacterial emboli are important in the production and localization of endocarditis.

Infectiousness of the Organism. Assuming that the susceptibility of the endocardium to infection is constant, bacterial infectability acquires considerable importance in determining whether or not bacterial endocarditis will develop. Three major factors that influence bacterial infectability are (1) the inherent virulence of the organism, (2) the intensity of exposure of the bacteria to

the endocardium, and (3) the duration of contact between bacteria and endocardium. The importance of each of these factors in the production of bacterial endocarditis has been well brought out by experimental data.

Virulence: Bacterial endocarditis can frequently be produced in normal dogs when Streptococcus hemolyticus is used [*24*], but when a less virulent organism (Str. mitis) is employed the addition of increased cardiac stress is required to obtain consistent results [*12*].

Intensity of exposure and duration of contact: In studying experimental endocarditis in dogs, Dick and Schwartz [*25*] noted that bacterial endocarditis could be more readily produced by increasing the dose of bacteria injected intravenously. The same investigators also learned that bacterial endocarditis could be more readily produced if the duration of the intravenous injection of a given dose of bacteria was prolonged.

The observation that right-sided bacterial endocarditis is three to five times more common than usual when the infection is acute and caused by unusually virulent organisms [*4,5,26,27*] serves to emphasize the effect that bacterial infectability has on the clinical incidence of right-sided bacterial endocarditis.

In passing, one should mention the concept that bacterial endocarditis selects left-sided structures because of the greater oxygenation of the blood on that side. This is not supported, however, by the extreme rarity of bacterial endocarditis in atrial septal defects [*20,21*] despite a high degree of arterialization of blood in the right atrium. In addition, oxygenated blood does not explain the greater frequency of bacterial vegetations at the pulmonary end than at the aortic end of the ductus in patent ductus arteriosus.

In the final analysis, the most important factor determining the site of localization of bacterial vegetations is probably local in the form of trauma.

MATERIAL AND METHOD

This study is based on twenty-three cases of bacterial endocarditis or endarteritis predominantly or exclusively involving the right side of the heart or pulmonary artery and obtained from the records of the Section of Pathologic Anatomy of the Mayo Clinic. The cases represent the period from 1911 through 1955. The salient clinical features of each case were analyzed from the clinical record. Whenever available, the gross specimens were re-examined, supplemental information being obtained from necropsy protocols. Histologic sections studied were usually those taken at the time of necropsy; additional sections were obtained whenever necessary. All sections were stained with hematoxylin and eosin. Elastic van Gieson and Brown-Gram stains were used for selected slides. Microscopic study was limited to the heart, lungs and kidneys. In nineteen instances bacterial vegetations were confined to the right side of the heart or pulmonary artery. Of the remaining four, minor vegetations appeared on the mitral valve, aortic valve or about the aortic ostium of a patent ductus arteriosus in addition to the larger right-sided vegetations.

CLINICAL OBSERVATIONS

Sex and Age. The percentile distribution between sexes approximated that for bacterial endocarditis in general [*5*], there being thirteen males and ten females. The average age was thirty-seven years, with a maximal age of seventy-two years and a minimal age of ten days.

It has long been apparent that bacterial endocarditis occurs infrequently in infants and young children [*28–30*]. In a recent review Macaulay [*31*] found in the literature only ninety-two cases of acute bacterial endocarditis occurring in children during the first two years of life, and to this group he was able to add thirteen cases of his own. Of particular interest was the relatively high incidence of tricuspid involvement, this valve bearing vegetations almost twice as frequently as the aortic valve. Three of the twenty-three cases presently under discussion involved infants less than two years of age. The patients were fifteen months,* four months and ten days old, respectively, at the time of death, and in each instance the tricuspid valve was the only site at which the vegetations localized.

Of further interest was the finding that a septic lesion of the skin was the probable portal of entry for bacteria in two of these three cases. Infantile eczema and epidermolysis bullosa were present in the fifteen month old and the ten day old patients, respectively. Micrococcus pyogenes was cultured from the blood of the first patient and from the skin lesions of the second. Unfortunately, no blood culture was obtained in the latter, but sections of the tricuspid valve contained many gram-positive cocci morphologically resembling M. pyogenes. Sites of infection

* This case has been the subject of a previous report [*32*] and is included in the five cases of isolated tricuspid endocarditis listed by Macaulay [*31*].

in the skin as a portal of entry for bacteria in endocarditis of infants have been emphasized by a number of authors [*33–35*]. Of the fifty-two cases in Macaulay's review in which a likely source of bacteria could be determined, skin sepsis appeared to represent the portal of entry in sixteen.

Thus the series presently reported includes three cases of isolated tricuspid endocarditis occurring before two years of age. To our knowledge, only four other such cases have been reported previously. The finding of skin sepsis as the probable source of infection and M. pyogenes as the likely etiologic agent in two of the three cases is not surprising, in the light of previous reports.

Duration of Disease. With six weeks as the dividing point, the disease had an acute clinical course in sixteen cases, and a subacute course in seven. That the clinical course of the infection is of very little value in suggesting the identity of the infecting organism is well brought out by an analysis of the cases of subacute infection. In the seven infections that fell into this category the etiologic organisms were as follows: Diplococcus pneumoniae in one, Neisseria gonorrhoeae in one, Streptococcus faecalis in one, Streptococcus viridans in one, an unidentified species of micrococcus in one, and Micrococcus pyogenes in two. In contrast, in the only other case in which the infection was due to Str. viridans the disease had a duration of only four and a half weeks from the onset of symptoms and would be classified as acute despite the fact that this organism usually causes a subacute infection. As stressed by Kelson and White [*36*], Str. viridans may cause a fulminating infection while usually virulent organisms may result in a lingering illness.

Symptomatology. Feverishness and chills were prominent features of the initial symptomatology in eighteen cases. In four instances dyspnea, and in five instances cough, were included among the presenting complaints. Pulmonary symptoms were present at some time during the illness in eighteen cases. These consisted of dyspnea in eight, thoracic pain in seven, cough in six, hemoptysis in three, and tachypnea in two.

Findings on Physical Examination. Fever was present in twenty-two cases. The remaining case was that of a four month old infant with tricuspid endocarditis who died from intracranial complications following a ventriculomastoid shunt for hydrocephalus. Perhaps the intracranial problem altered the usual thermal response to infection.

Cardiac murmurs were noted in only eight cases. A review of 200 cases of bacterial endocarditis by Parsons and associates [*37*] at the Mayo Clinic revealed that a murmur was heard at one time or another during the illness in all patients. The frequency of acute infections and tricuspid endocarditis in the present series, in addition to the fact that a diagnosis of endocarditis in this study was based on necropsy data rather than on clinical data, probably explains the marked discrepancy in the two series.

A cardiac murmur was present in only three of the fifteen cases of tricuspid endocarditis, and in none was it loudest at the tricuspid area. Cardiac murmurs characteristically have been inconstantly present and often totally lacking in tricuspid endocarditis. In only eight of the twenty cases of tricuspid endocarditis reported by Goldburgh and associates [*38*] was there a murmur, and in none of these was it heard specifically over the tricuspid area. In the series of heroin addicts with tricuspid endocarditis reported by Hussey and Katz no positive evidence of tricuspid involvement was noted [*39*]. Nevertheless, the lesion was often strongly suspected when signs of right-sided endocarditis were unassociated with any murmur indicative of pulmonary valvular disease. This is further substantiated by the findings in this study and indicates that one of the cardinal diagnostic features of bacterial endocarditis, namely a cardiac murmur, is usually absent when tricuspid endocarditis is being considered.

In only two of the five cases in which there were vegetations on the pulmonary valve was a cardiac murmur noted, but in both instances it was located over the pulmonic area. In one case, however, the murmur had apparently been present many years previously when a diagnosis of congential heart disease had first been made. The heart at necropsy showed congenital stenosis of the pulmonary valve. Why no murmur was noted in the other three cases is hard to explain since the pulmonary vegetations were of substantial size in each. Nevertheless, it previously was observed [*40*] that subacute bacterial endocarditis involving the pulmonary valve frequently is not associated with murmurs.

In the two cases of infected patent ductus arteriosus the typical "machinery" murmur of that condition was heard and the disease was readily diagnosed antemortem. A loud systolic

murmur and thrill were present at the base in the single case* of right-sided bacterial endocarditis complicating a ventricular septal defect.

Positive physical findings referable to the *thorax* were noted in eight of the twenty-three cases. Dullness was present and breath sounds were decreased in five cases, rales were present in four, cyanosis in two, a pleural rub in two and limitation of thoracic expansion in one case.

The *spleen* was readily palpable in six cases and questionably palpable in two others. In one of the cases in which there was clinical splenomegaly and in one in which the spleen was questionably palpable there were small left-sided vegetations as well.

Definite evidence of *peripheral embolization* with vascular occlusion was present in three cases. In each instance there was a potential source of emboli on the systemic side of the circulation. In two cases small vegetations were present on aortic and mitral valves while in the third case there were minute vegetations at the aortic end of a patent ductus. No peripheral embolization occurred in the other case in which left-sided vegetations were present.

When gross peripheral emboli do form in isolated right-sided endocarditis they are most likely to be related to pulmonary venous thrombosis secondary to pulmonary infarction.

Petechiae were present in only three cases in this series. *Clubbing* was not found in any.

Multiple abscesses developed in various areas supplied by the peripheral circulation in nine of the twenty-three cases. The most common locations were the subcutaneous tissue and kidneys. In two cases the infecting organism was not identified, but in all other instances in whch multiple abscesses were present M. pyogenes var. aureus was the etiologic organism.

Laboratory Findings. Blood cultures were obtained in eighteen cases and the results were positive in all but one. When the four cases in which lesser vegetations were present on the left side of the heart, or at the aortic end of a patent ductus, are eliminated, the frequency of positive blood cultures in isolated right-sided endocarditis or pulmonary endarteritis is still high—93 per cent.

This figure seems surprisingly high in view of Blumgart's [*43*] observation that infection of the peripheral blood stream is frequently absent when subacute bacterial endocarditis is confined to the right chambers of the heart. In addition, Barker's [*44*] study of right-sided subacute bacterial endocarditis revealed that blood cultures gave positive results in only 62 per cent of the cases in which cultures were made. This discrepancy may be explained by the large number of acute infections in our series. It is conceivable that in fulminating and overwhelming infections the so-called filtering action of the lungs becomes inadequate to prevent bacteria from reaching the systemic circulation in sizable numbers. In addition, the pulmonary infection may proceed at a more rapid rate and provide a ready source from which organisms can enter the systemic circulation.

Of the five cases in which blood cultures were not obtained, M. pyogenes (staphylococcus) was grown in a spinal fluid culture from one, while in another Escherichia coli and M. pyogenes were obtained from cultures of the skin. In the latter case histologic sections of endocardial vegetations revealed organisms with the morphologic appearance of M. pyogenes. Although not conclusive, it seems highly probable that M. pyogenes was the etiologic agent in both of these cases, and in subsequent discussion will be treated as such.

The causative organism was identified in nineteen of the twenty-three cases. The bacteria were variously identified as "Staphylococcus aureus," "staphylococcus" or "M. pyogenes" in eleven of these nineteen, so that they probably represented M. pyogenes in each instance. Of the remainder, three were caused by D. pneumoniae, two by Str. viridans and one each by N. gonorrhoeae, Str. hemolyticus and Str. faecalis.

At first glance it is surprising to find M. pyogenes the infecting organism in over more than half the cases in which the causative organism was identified. Of the twenty-four cases of right-sided endocarditis in Thayer's [*5*] series there was a relatively even distribution among four different bacteria, namely M. pyogenes var. aureus, N. gonorrhoeae, streptococcus and D. pneumoniae.

With the advent of chemotherapy and antibiotics, however, pneumococcal and gonococcal infections had become much more amenable to therapy, and the incidence of streptococcal infections has decreased as well. Because of its remarkable ability to develop resistant strains

* This case has been reported previously [*41,42*].

[*45,46*], however, M. pyogenes has continued to be a difficult organism to combat. With this in mind, the cases were grouped according to whether or not antibiotic therapy had been used. Penicillin was first used in this series in 1945, and in all subsequent cases various antibiotics were used. Of the infections occurring before 1945, 45 per cent were caused by M. pyogenes. The percentage increased to 75 per cent when infections occurring after 1945 were considered. Although the number of cases is inadequate to permit conclusions to be drawn, M. pyogenes appears to have become by far the commonest cause of right-sided bacterial endocarditis over the past decade.

D. pneumoniae was responsible for right-sided bacterial endocarditis in three cases (16 per cent). This is considerably less than the 45 per cent found by Goldburgh and co-workers [*38*] in their study of acute tricuspid endocarditis. Part of this discrepancy may be related to the use of penicillin, as all three cases occurred prior to the availability of this antibiotic.

Both infections caused by a non-hemolytic species of streptococcus were superimposed on congenital cardiac anomalies. Since non-hemolytic species of streptococcus are relatively avirulent, it is not surprising to find them associated with lowered endocardial resistance to infection in the form of congenital heart disease.

One case of tricuspid endocarditis due to Str. hemolyticus occurred following a postoperative wound infection.

Str. faecalis produced tricuspid endocarditis in one case, with lesser vegetations on the mitral and aortic valves. The duration of this illness, eight months, was the longest in the series. No source of infection or pre-existing valvular disease could be detected.

Gonococcal endocarditis occurred once and involved the pulmonary valve principally and the mitral valve to a lesser extent. Thayer [*5,47*] expressed the view that involvement of the pulmonary valve is rather characteristic of gonococcal endocarditis.

The marked preponderance of infections due to usually virulent organisms is not surprising. As already pointed out, the frequency of right-sided endocarditis is greater when virulent organisms are the etiologic agents.

A *leukocyte count* greater than 10,000 per cu. mm. of blood was recorded in fifteen of the nineteen cases in which a count was obtained. The majority ranged between 10,000 and 25,000 per cu. mm.; in one case a count of 56,000 per cu. mm. was recorded.

In the evaluation of *anemia*, one case associated with erythroblastosis fetalis and one associated with menometrorrhagia were excluded. There remained fourteen cases in which the hemoglobin concentration or the erythrocyte count or both were determined during the course of the infection. On the assumption that a hemoglobin concentration of less than 12 gm. per 100 cc. of blood in males or less than 11 gm. per 100 cc. in females is indicative of anemia, there were eleven cases (79 per cent) in which anemia occurred. This correlates very well with the presence of anemia in 75 per cent of the patients with bacterial endocarditis studied by Parsons and associates [*37*].

After exclusion of a case in which there was chronic glomerulonephritis, there were seventeen cases in which the results of *urinalysis* were available. Significant abnormalities were present in twelve of these. The commonest of these was pyuria, which occurred in fifteen cases. Albuminuria was present in twelve cases and microscopic hematuria in nine. Casts were demonstrable in only three of the seventeen cases.

The concentration of *blood urea* was in excess of 40 mg. per 100 cc. in nine of twelve cases in which there was no pre-existing renal disease.

The *sedimentation rate* was abnormal in four cases, normal in two and not determined in seventeen.

Thoracic Roentgenograms. A roentgenogram of the thorax was made at some time during the illness in seventeen of the twenty-three cases. In all but three there was some evidence of pulmonary disease. The commonest roentgenographic interpretation was pulmonary infiltration or pneumonitis.

Predisposing Factors. Evidence of pre-existing *rheumatic valvulitis* was present in only one instance. The heart was that of a seventy-two year old man in whom symptoms of bacterial endocarditis developed six days after ureteral manipulation for a renal stone. Preoperatively he had a loud apical systolic and a soft apical diastolic murmur. At necropsy the heart weighed 775 gm. and there was slight fusion of the aortic valve cusps at their commissures. The mitral, and to a lesser degree the tricuspid valve, showed thickening and shortening of the chordae tendineae.

Despite associated aortic and mitral rheumatic valvular disease, the tricuspid valve was the one in which a vegetation formed.

Congenital anomalies were present in four of the twenty-three hearts studied. Patent ductus arteriosus occurred twice, congenital stenosis of the pulmonary valve occurred once, and there was one case of ventricular septal defect with double tricuspid orifice. This incidence (17 per cent) is considerably lower than the incidence of 61 per cent found by Barker [*44*] in studying right-sided subacute bacterial endocarditis and may be related to the high incidence of acute bacterial endocarditis in our group. Although the etiologic bacterial agent was not identified in many of Barker's cases, Str. viridans was undoubtedly more often responsible in his series than in ours.

The occurrence of bacterial vegetations at the probable location of a jet lesion on the anterior wall of the right ventricle and septal leaflet of the tricuspid valve in the case in which there was a ventricular septal defect, and the localization of vegetations at the pulmonary end of the ductus in the hearts from the two cases of patent ductus arteriosus, are particularly good examples of the influence *trauma* has on localizing bacterial vegetations.

As already indicated, *virulence* appears to have played a major role in the development of right-sided bacterial endocarditis in the cases included in this study. In seventeen of the nineteen cases in which a causative organism was identified the causative bacteria were considered to be of a relatively virulent variety.

Portal of Entry for Bacteria. The portals through which bacteria gained access to the circulation in nineteen cases are as follows: surgical procedures, ten; skin diseases, three; respiratory infections, three; and prostatic massage, urethritis, dental infection, one each. In four cases no obvious portal of entry was noted. It is significant that all but two cases of M. pyogenes endocarditis followed either a surgical procedure or a skin infection. The importance of this organism as a secondary invader recently has been stressed by Rogers [*46*], who also noted a correlation between denuding skin disease and M. pyogenes bacteremia. As might be expected, all cases of pneumococcal endocarditis followed a respiratory infection.

No portal of entry was apparent in the cases of Str. viridans endocarditis. This may be related to the observation that the organism is a common cause of transient bacteremia originating from oral sepsis [*48*]. Such a portal of entry could be easily overlooked.

PATHOLOGIC OBSERVATIONS

Size of Heart. There was no definite evidence that right-sided endocarditis *per se* produced significant cardiac hypertrophy.

Location of Vegetations. The tricuspid valve was predominantly involved in fifteen of the twenty-three cases. In two instances there were lesser vegetations on the aortic and mitral valves as well. Vegetations were predominantly on the pulmonary valve of the heart in five cases. In one of these the vegetation was superimposed on a cardiac anomaly in the form of congenital pulmonic stenosis. In another instance there was a small vegetation on the mitral valve as well.

There were two cases in which there was infection of a patent ductus arteriosus. In both instances the vegetations were mostly about the pulmonary end of the ductus. Because of traumatic factors [*49*] this is the characteristic location of vegetations in this condition. In one case minute vegetations were also located at the aortic ostium of the patent ductus. Microscopic section showed disruption and fragmentation of the elastic tissue of the pulmonary artery about the ductus, and in one case a small aneurysmal dilatation of the pulmonary artery had occurred at this site. As noted by Schlaepfer [*50*], this is not an infrequent occurrence.

The main feature of the one case in which there was a ventricular septal defect is that vegetations occurred at the two usual sites of trauma from the anomalous flow of blood through the ventricular septal defect. These are the septal leaflet of the tricuspid valve and the anterior wall of the right ventricle.

The myocardial lesions of subacute bacterial endocarditis have been carefully studied by Perry and associates [*51*]. Microscopically they may be divided into focal and diffuse types. Focal lesions may take the form of focal myocarditis, abscesses, microinfarcts and lesions of the myocardial vessels. The diffuse lesions consist of widespread interstitial collections of neutrophils and macrophages.

It has been suggested that bacteria, emboli or their products must have direct access to the coronary circulation before myocardial lesions will result, and that no myocardial involvement should be present in cases in which vegetations are confined to the right side of the heart. The

results of the present study substantiate this concept to some extent. Microinfarcts of the myocardium were noted in only one instance. This was in a case of M. pyogenes endocarditis of the tricuspid valve in which additional smaller lesions were present on the aortic and mitral valves as well.

In only six of the nineteen cases in which vegetations were confined to the right side of the heart were there lesions in the myocardium. Small foci of perivascular polymorphonuclear leukocytes were present in two cases, minimal diffuse interstitial infiltration with polymorphonuclear leukocytes in two, acute myocarditis of the right atrium associated with pericarditis in one, and miliary abscesses containing an occasional aggregate of bacteria in one. The absence of microinfarcts in this group suggests that the lungs are much more effective in filtering out minute emboli than they are in preventing bacteria from entering the coronary circulation. This correlates well with the high incidence of positive blood cultures and miliary abscesses plus the rarity of embolic peripheral arterial occlusion in the cases under discussion.

Lungs. In twenty-one cases septic pulmonary embolism of varying degrees occurred. The complications of this phenomenon were infarction, pneumonia and abscess formation, plus thrombosis and septic arteritis of the branches of the pulmonary artery.

Spleen. In six of the twenty-three cases the spleen was definitely palpable, and in two additional cases it was questionably palpable. In one of the two a mass was felt in the left upper quadrant and the patient was subsequently found to have an abscess in the tail of the pancreas, and in addition, a markedly enlarged spleen. Which of these was felt clinically it is impossible to say. Splenomegaly as determined at necropsy occurred in 77 per cent of cases.

Kidney. Renal lesions related to bacterial endocarditis were encountered in fourteen of the twenty-three cases. These could be grouped into five major categories, namely (1) suppurative nephritis, focal or diffuse, (2) glomerular and tubular hemorrhage, (3) acute diffuse proliferative glomerulonephritis, (4) focal "embolic" glomerulonephritis, and (5) infarction.

Suppurative nephritis: This term implies the reaction of the kidney to direct contact with the infecting bacteria which took the course of either focal abscess formation or a diffuse suppurative process involving the renal parenchyma and interstitial tissue in a manner not unlike that of pyelonephritis.

Suppurative nephritis, the commonest renal manifestation encountered, was present in eight cases. In an additional case there was mild interstitial inflammation of the kidney with lymphocytic infiltration the exact significance of which is uncertain.

The causative organism was identified in six of the eight cases of suppurative nephritis, and in all but one of these M. pyogenes was the bacterium involved; in the one case the causative organism was a species of Str. hemolyticus and the renal lesion was that of severe, acute, diffuse, exudative nephritis without abscess formation.

In one case in which the renal disease was classified as suppurative nephritis the renal lesion was that of acute necrotizing papillitis. The patient had been diabetic and the kidney manifested advanced changes of Kimmelstiel-Wilson's disease as well.

Glomerular and tubular hemorrhage: Glomerular and tubular hemorrhage without definite inflammatory reaction occurred in two of the twenty-three cases. D. pneumoniae and Str. faecalis were the causative organisms. It is possible that these findings represented an initial stage of acute diffuse proliferative or focal "embolic" glomerulonephritis, but there was insufficient evidence to establish either diagnosis.

Acute diffuse proliferative glomerulonephritis: Primary, acute, diffuse, proliferative glomerulonephritis did not occur in this series. In one instance of tricuspid endocarditis, chronic glomerulonephritis had been present for at least five years prior to death. Nevertheless, many glomeruli had been spared and at necropsy these showed the changes of acute, diffuse, proliferative glomerulonephritis.

Focal embolic glomerulonephritis: There were two cases in which the characteristic lesions of focal embolic glomerulonephritis were present. One was a case of pneumococcal endocarditis confined to the tricuspid valve, while the other was a case of gonococcal endocarditis predominantly of the pulmonary valve, but with lesser vegetations on the mitral valve as well.

It is of interest to find focal embolic glomerulonephritis produced by pneumococcal and gonococcal endocarditis. Usually these renal lesions are associated with Str. viridans or occasionally with Str. hemolyticus [*52*] and Hemophilus influenzae [*53*] infections. In addition, they were previously considered to be small infarcts of

embolic origin [*54–56*], thus prompting the present nomenclature. More recently this etiologic explanation has been challenged and they are now thought by some to represent either an intrinsic fibrinoid reaction presumably in response to an immuno-allergic mechanism [*57*], thrombotic in nature [*52*], or a local vascular inflammation [*58*]. Because of this, Allen [*57*] has even discarded the term "focal embolic glomerulonephritis" in favor of "focal endocarditic glomerulonephritis."

The finding of one case of bacterial endocarditis confined to the right side of the heart and associated with focal embolic glomerulonephritis tends to support the theory that these lesions are not the result of minute emboli originating from the infected valve leaflet. Bell [*59*] has also observed focal embolic glomerulonephritis in three cases of endocarditis limited to the right side of the heart.

M. pyogenes endocarditis, despite its high incidence in this series, was not associated with focal embolic glomerulonephritis. This agrees with the previous observations of Bell [*60*].

Infarction: No gross renal infarcts were present. In one case of suppurative nephritis associated with M. pyogenes endocarditis predominantly of the tricuspid valve, but with lesser vegetations on the aortic and mitral valves as well, there was a microinfarct distal to a small renal artery which had been occluded by a septic embolus.

CLINICAL DIAGNOSES

A clinical diagnosis of endocarditis or endarteritis was made in only six of the twenty-three cases, and in only three instances was the correct location of the vegetations determined antemortem. A cardiac murmur was the single most important finding that assisted the clinician in making an accurate diagnosis, its absence was probably his greatest hindrance. All six cases in which the diagnosis of endocarditis or endarteritis had been made were associated with obviously organic cardiac murmurs and positive blood cultures. In the three cases in which a correct diagnostic localization of the vegetations was made the murmurs were characteristic of the type of defect involved; in two of these the typical machinery murmur of patent ductus arteriosus was present, and in the presence of fever and a positive blood culture a diagnosis of infected patent ductus was readily made; in the third case a murmur quite typical of pulmonic stenosis was present which, with a positive culture, led to a diagnosis of bacterial endocarditis of the pulmonary valve. In the remaining three cases in which an antemortem diagnosis of bacterial endocarditis was made there were obvious murmurs but no attempt was made to localize the site of the lesion; the presence of pulmonary symptoms, physical findings in the lungs, and changes manifested in the thoracic roentgenogram in one of these cases should perhaps have strongly suggested to the clinician that the vegetations were right-sided in location.

In no instance was a diagnosis of bacterial endocarditis made in cases in which there was no significant cardiac murmur. Since fifteen of the twenty-three cases in this series did not present this finding, the absence of a cardiac murmur and the resultant hindrance to the establishment of a diagnosis in right-sided endocarditis assume considerable importance. The fact that 80 per cent of the cases without murmurs were those of tricuspid endocarditis indicates that this localization causes the most difficulty in diagnosis.

In an effort to determine the diagnostic value of various clinical features commonly associated with bacterial endocarditis, the frequency with which each occurred in the fifteen cases without cardiac murmurs was determined. The following results were obtained: fever in 93 per cent, leukocytosis in 92 per cent, pulmonary symptoms or findings in 87 per cent, anemia in 75 per cent, positive blood cultures in 73 per cent, hematuria in 70 per cent, and splenomegaly in 7 per cent. It is apparent that signs of infection, anemia, positive blood cultures, microhematuria, and evidence of pulmonary infarction without disease in peripheral veins should always make the diagnosis of right-sided endocarditis come to mind, despite the absence of significant cardiac murmurs.

APPARENT CAUSE OF DEATH

By far the commonest cause of death in this group was extensive pulmonary infarction and generalized sepsis. This was true in seventeen of the twenty-three cases.

In only two instances did cardiac failure appear to play a significant role in the fatal outcome. In both, pulmonary infarction was present as well, and in one of these, bouts of congestive cardiac failure secondary to hypertensive and rheumatic heart disease had occurred prior to the bacterial endocarditis.

In both cases of patent ductus arteriosus, death resulted from massive hemorrhage at

operation during attempted closure of the ductus. As pointed out by Touroff [*61*], this is the principal hazard in surgical treatment of patent ductus arteriosus complicated by subacute bacterial endocarditis.

In one of the two remaining cases, death resulted from massive pulmonary hemorrhage presumably secondary to erosion of a branch of the pulmonary artery by septic arteritis. In the other case death followed subdural hemorrhage and cerebellar infarction complicating a ventriculomastoid shunt for hydrocephalus.

COMMENT

Right-sided bacterial endocarditis is an uncommon clinical entity. Although a number of factors may be implicated in explaining this low incidence, it would seem that the lesser local trauma to the endocardium of the right side of the heart is particularly important in this regard. The influence of local trauma in the localization of vegetations in bacterial endocarditis is specifically demonstrated in certain congenital cardiac malformations. "Jet lesions" produced by the impingement of an anomalous stream of blood on the wall of the right ventricle or septal leaflet of the tricuspid valve in ventricular septal defects, or those found on the wall of the pulmonary artery opposite a patent ductus arteriosus, are common sites for bacterial vegetations to localize in cases of right-sided endocarditis. The predilection of bacterial vegetations to develop on the narrowed pulmonic end of a patent ductus arteriosus and on the pulmonary valve in congenital pulmonic stenosis are additional examples. It thus seems logical to consider local trauma an important factor in determining the site of localization of bacterial endocarditis in the more common situations.

Two factors appear to be of particular importance in subjecting the left side of the heart to a greater degree of local trauma than the right side. One is the greater frequency of deforming lesions like rheumatic valvulitis and bicuspid aortic valve on the left side, and the other is the higher intracardiac pressures found on the left. In one instance local trauma may be produced by the disturbed mechanics associated with valvular deformity, and in the other it may be associated with the relatively high hemodynamic pressures.

As previously discussed, there are undoubtedly other factors such as resistance of the host, endocrine imbalance and bacterial virulence, in addition to intensity and duration of bacterial exposure, which play varying roles in the final development of bacterial endocarditis. None seem of major importance at the present time in establishing the preference of bacterial endocarditis for the left side of the heart.

From the material in this study there appears to be no significant difference in sex distribution between right-sided bacterial endocarditis and endocarditis in general. The finding of three cases in which the age was less than two years suggests a somewhat higher incidence of right-sided endocarditis in infants.

Right-sided bacterial endocarditis is more apt to occur as an acute than as a subacute infection. Pulmonary symptoms often associated with chills and fever are the most common presenting symptoms.

Cardiac murmurs are present in less than half the cases of right-sided endocarditis. The diagnostic value of a cardiac murmur is particularly limited in tricuspid endocarditis, but its absence may also be misleading in pulmonic endocarditis. Murmurs are most often present when there is an associated congenital cardiac anomaly.

Clinical splenomegaly occurs in about 25 per cent of cases.

Apart from metastatic abscesses and petechiae, peripheral embolization is extremely uncommon in isolated right-sided bacterial endocarditis.

Although the lungs are efficient in preventing embolic particles from entering the systemic circulation, their effectiveness in filtering out bacteria is very limited. This was reflected in the frequency of positive blood cultures and the relatively high incidence of metastatic abscesses in the cases studied, despite the absence of peripheral embolization with vascular occlusion in all cases of isolated right-sided endocarditis.

M. pyogenes is the commonest cause of right-sided bacterial endocarditis. This has become particularly true following the advent of antibiotic therapy. When a non-hemolytic species of streptococcus is the infecting organism, the infection is often superimposed upon a pre-existing congenital cardiac anomaly or an acquired valvular deformity.

Evidence of pulmonary disease is commonly found in the thoracic roentgenogram. The leukocyte count and erythrocytic sedimentation rate reflect the presence of systemic infection. Anemia and abnormalities of the urine are also frequently encountered in right-sided endo-

carditis. The concentration of blood urea is often increased as well.

Cardiac enlargement is seldom demonstrable in right-sided endocarditis without antecedent cardiac disease. The tricuspid valve is the commonest location for vegetations on the right side of the heart. This site is three times as common as the pulmonic valve. Congenital cardiac anomalies most often associated with right-sided bacterial endocarditis are patent ductus arteriosus, ventricular septal defect and pulmonic stenosis. Although a representative example was not present in this series, right-sided endocarditis associated with the tetralogy of Fallot should be included.

Myocardial lesions are infrequent, while pulmonary embolization with resultant septic infarction is almost a constant finding with bacterial vegetations on the right side of the heart.

Suppurative nephritis is the most common renal manifestation, particularly when M. pyogenes is the infecting organism. Glomerular and tubular hemorrhage and focal embolic glomerulonephritis may also occur. The finding of the latter with endocarditis confined to the right side of the heart makes the embolic origin of these lesions seem unlikely.

SUMMARY

A study of the clinical and pathologic features of twenty-three cases of bacterial endocarditis encountered at the Mayo Clinic in the period from 1911 through 1955, in which the right side of the heart or pulmonary artery was predominantly involved, disclosed the following points of significance:

In nineteen cases the vegetations were exclusively right-sided in location, while in the four remaining cases there were lesser vegetations also present on the left.

The clinical course was usually acute. The results of blood cultures were positive in seventeen of eighteen patients from whom specimens were obtained. The causative organism was identified by other means in two additional cases. Among the nineteen cases in which bacteria were isolated, the endocarditis was due to M. pyogenes eleven times, D. pneumoniae three times, Str. viridans twice and N. gonorrhoeae, and Str. hemolyticus and Str. faecalis once each. Following the advent of antibiotics the proporton of the infections due to M. pyogenes increased from 45 per cent to 75 per cent.

Cardiac murmurs were noted less often than in left-sided endocarditis, being detected in only 35 per cent of the cases. Other features of bacterial endocarditis, such as signs of infection, anemia, positive blood cultures and microhematuria together with evidence of pulmonary infarction should suggest right-sided vegetations despite the lack of a cardiac murmur.

Tricuspid endocarditis represented 65 per cent of the total cases. Palpable splenomegaly and peripheral embolization were less frequent than with bacterial endocarditis in general. Septic pulmonary infarction was an almost constant finding, and renal lesions often occurred.

In the majority of instances right-sided bacterial endocarditis followed some operative procedure or some other obvious source of infection such as cutaneous sepsis. When it appeared to develop spontaneously there was usually a pre-existing valvular deformity and Str. viridans was the responsible organism.

Death usually resulted from extensive pulmonary infarction and septicemia.

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Seminar on Liver Disease

Bilirubin Metabolism in Jaundice*

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OUR earliest records of jaundice date back to 400 B.C. when Hippocrates described it. At the end of the second century A.D., Galen associated jaundice with the presence of stones in the biliary tract, and about 1,000 A.D. Avicenna, in his Canon, discriminated between jaundice due to obstruction and other kinds of jaundice. The association of bile pigments with haemoglobin was demonstrated by Virchow [*1*], who described the formation of bilirubin crystals (haematoidin) in blood extravasates. In 1874 the conversion of haemoglobin to bile pigments was established by the experiments of Tarchanoff [*2*], in which he showed that the introduction of haemoglobin into the vascular circulation of dogs with biliary fistula resulted in an increased excretion of bile pigment.

There has been much speculation about the mechanisms involved in the conversion of haemoglobin to bilirubin, and haematin and protoporphyrin have been suggested as possible intermediate compounds [*3,4*]. The literature has recently been reviewed by Lemberg [*5*] and he favours the theory that the porphyrin ring in the haemoglobin molecule is opened before the iron is removed, while the tetrapyrrolic pigment is still coordinated with globin. The resulting pigment, choleglobin, is then converted to biliverdin, which is reduced to bilirubin. It remains to be seen whether or not such a mechanism participates in normal haemoglobin metabolism for the evidence is circumstantial and, although the presumed intermediates can be recognised spectroscopically, they have not been isolated in a sufficiently pure state for thorough examination.

The pigment which is excreted in the bile undergoes further reduction in the gut and appears in the faeces as stercobilin. Isotope experiments, involving the isolation of labelled stercobilin after feeding ^{15}N glycine, show that in normal people about 80 per cent of the stercobilin comes from the breakdown of haemoglobin from mature erythrocytes at the end of their life span. A proportion of the ^{15}N stercobilin, which may be as high as 80 per cent in cases of pernicious anaemia or porphyria, is excreted immediately after glycine feeding; this may either come from the breakdown of immature erythrocytes in the bone marrow, from haem synthesised in excess of globin, or from tetrapyrrol intermediates formed during haemoglobin synthesis [*6,7*]. The possibility of alternative routes of bilirubin synthesis must therefore be borne in mind when considering the problems of bilirubin metabolism, particularly in relation to pathological conditions.

Role of the Liver. The problem as to whether the liver merely excretes bilirubin, which was formed elsewhere, or alternatively forms bilirubin in addition to excreting it, occupied many workers at the turn of the century [*8*]. However, once successful hepatectomy had been achieved in mammals [*9,10*], it was established that the formation of bilirubin from the breakdown of haemoglobin took place in the reticuloendothelial system and that the bilirubin is then transported in the plasma to the liver which excretes the pigment in the bile.

In 1913 van den Bergh and Snapper [*11*] devised a much needed method for the quantitative estimation of bilirubin in plasma by adapting Ehrlich's highly specific test for bilirubin in urine. In this method, which is the basis of all methods used today, alcohol was used to provide a homogeneous medium in which bilirubin and the coupling agent are both soluble. Van den Bergh and Müller [*12*] found by an accidental omission that alcohol was not necessary for colour development when bile was used

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as a source of pigment, and that a *direct* reaction was also given by plasma from patients with obstructive jaundice and hepatitis. Alcohol was, however, necessary for colour development with normal (horse) plasma or plasma from patients with haemolytic jaundice, when there was no evidence of obstruction; such a reaction has been called *indirect* and the pigment giving this reaction has been referred to as the "indirect reacting pigment," in contrast to the "direct reacting pigment." It thus became apparent that the liver produces some change in the bilirubin in the course of its passage into the bile ducts or, if these are blocked, when returning it to the blood.

TABLE I
DIFFERENCES BETWEEN THE TWO TYPES OF BILE PIGMENT

Property	Bilirubin	Conjugated Bilirubin
Van den Bergh reaction	Indirect	Direct
Solubility in aqueous solution at acid or neutral pH	−	+
Solubility in chloroform and lipid solvents	+	−
Occurrence in icteric urine	−	+
Occurrence in bile	−	+
Affinity for brain tissue	+	−
Attachment to plasma albumin	+	+
Affinity for denatured plasma proteins	−	+
Ease of oxidation	+	++
Photosensitivity	++	+
Association with haemolytic jaundice	++	(+)
Association with obstructive jaundice and hepatitis	+	+++

The differences between "indirect" and "direct" bilirubin are summarised in Table I. Much time, energy and paper has been spent in endeavouring to explain these differences [*3,13*]. Some investigators considered that there were two forms of bilirubin while others thought that, in serums giving an indirect reaction, bilirubin was chemically bound with protein or some other substance so that alcohol was necessary to liberate the bilirubin before it could react with the coupling agent (diazotised sulphanilic acid). Klatskin and Bungards [*14*] have recently re-examined the distribution of the two types of bile pigment among the different plasma proteins. They agree with Gray and Keckwick [*15*] and Martin [*16*], who used electrophoretic technics, that both pigments are predominantly attached to albumin. The possibility that the direct reaction is due to an association of bilirubin with bile acids has been suggested [*17*], but it has now been found that this was due to incomplete separation of the pigment and bile acids so that this explanation is untenable. Yamaoka and his Japanese colleagues [*19*] have proposed an ester structure for direct bilirubin; they also consider that some of the direct pigment is a salt-form of bilirubin.

Identification of "Direct Bilirubin." In 1953 Cole and Lathe [*20*] reported the separation of the two types of pigments, which reacted directly and indirectly in the van den Bergh reaction. Both fractions were free of protein. This separation was achieved using reverse phase partition chromatography on silicone-treated kieselguhr and a solvent system of chloroform, methanol, carbon tetrachloride and pH 6 buffer solution. At about the same time workers from Japan [*21*], France [*22*] and Germany [*23*] obtained a similar separation using paper chromatography.

The more water-soluble pigment, which moved faster in the Cole and Lathe system, predominated in the serum of obstructive jaundice and bile and was observed to be a mixture of two pigments (pigments I and II). They were separated by reverse phase chromatography using a solvent system of n-butanol and pH 6 buffer solution [*24*]; both pigments reacted directly in the van den Bergh reaction. The more water soluble of these pigments, pigment II, is the dominant pigment in human bile, while both pigments I and II are found in urine and plasma from patients with obstructive jaundice and hepatitis. In haemolytic jaundice, the slow moving indirect reacting pigment, which has been identified as bilirubin, is the chief bile pigment. The unstable nature of pigments I and II has so far prevented their isolation, and their characterisation has only been possible by indirect means. The similarity of their absorption spectrums shows that they are closely related to each other and to bilirubin. Unlike bilirubin, they are soluble in aqueous solution at an acid or neutral pH and insoluble in chloroform.

A study of the more stable azo pigments formed in the van den Bergh reaction by bilirubin, pigment I and pigment II, has provided many of the clues necessary for the identification

of direct bilirubin. Billing [*25*] separated these pigments on a partition chromatogram and found that serum from obstructive jaundice produced two azo pigments (pigments A and B). A similar observation was made independently by Kawai [*21*] using a paper chromatographic technic. Billing showed that pigment II gives rise to pigment B, which is more water soluble than pigment A formed from bilirubin, while pigment I produces a mixture of pigments A and B. (Fig. 1.) (Because of the unsymmetrical arrangement of the β side chains in the bile pigments, pigments A and B must each consist of a mixture of two isomers, differing only in the position of the methyl and vinyl side chains. These isomers can be separated on partition chromatograms but as they behave very similarly they will be discussed as if they were one substance.)

It seemed probable that the difference in structure between bilirubin and pigment II was similar to that between pigment A and pigment B. The structure of pigment A had been established by Fischer and Haberland [*26*]. Billing, Cole and Lathe [*27*] prepared a specimen of pigment B (87 per cent pure) from diazotised human bile, using a series of countercurrent procedures. Pigment B was identified as the monoglucuronide of pigment A as the result of enzymatic and chromatographic studies and quantitative determinations of glucuronic acid content. The ease with which mild alkali treatment converted pigment B to pigment A was in favour of an ester linkage between the propionic acid side chain of pigment A and the C_1 hydroxyl of glucuronic acid, rather than a glycosidic linkage. Evidence that pigment B is the glucuronide of pigment A has also been presented by Schmid [*28*], who purified pigment B using paper chromatography.

The work of Fischer and Haberland [*26*] indicated that the coupling of bilirubin with diazo reagents involved a preliminary hydrolysis at the methene bridge. They said that only one of the resulting dipyrrol compounds reacted with the diazonium salt to give azobilirubin (pigment A). Overbeek, Vink and Deenstra [*29*] extended these observations to show that, in the presence of sufficient coupling agent, a second and slower reaction occurred in which the other half of the bilirubin molecule, a hydroxypyrromethene carbinol, also coupled with the diazonium salt. Two isomeric molecules of pigment A are therefore formed from one molecule of bilirubin. (Fig. 2.) Since pigment II forms only pigment B in this reaction it is possible to conclude, by analogy, that one molecule of pigment II will form two isomeric molecules of pigment B. If pigment B is the monoglucuronide of pigment A then it follows that pigment II is the diglucuronide of bilirubin.

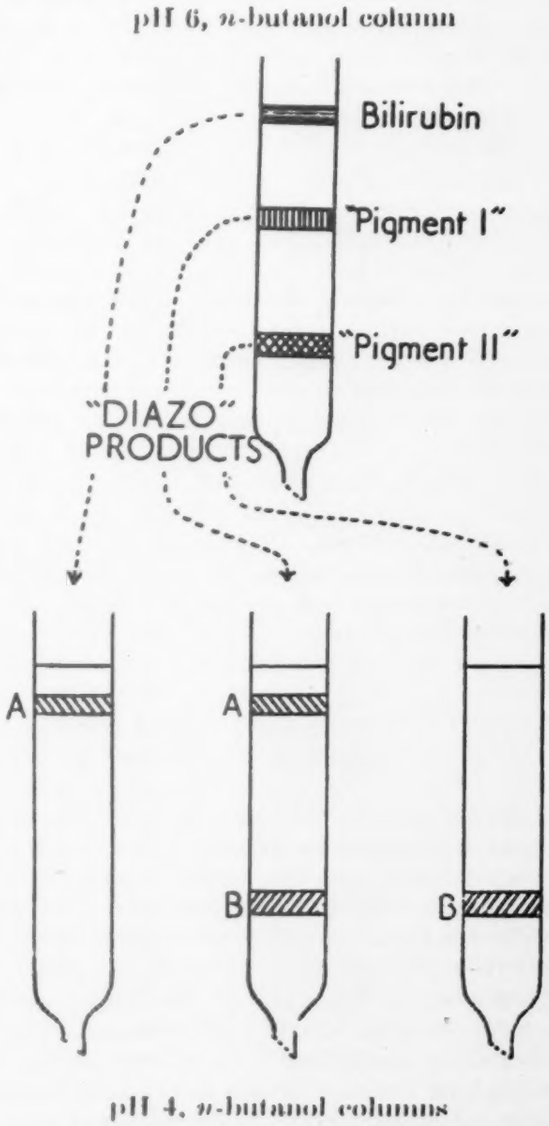

FIG. 1. Behaviour of plasma bile pigments and their diazo products on partition chromatograms. LATHE, G. H. Biochemical Society Symposia No. 12, p. 34, 1954.

Talefant [*30,31*], using a more direct approach to the problem, has also concluded that in direct bilirubin, bilirubin is probably bound to glucuronic acid. By means of paper electro-

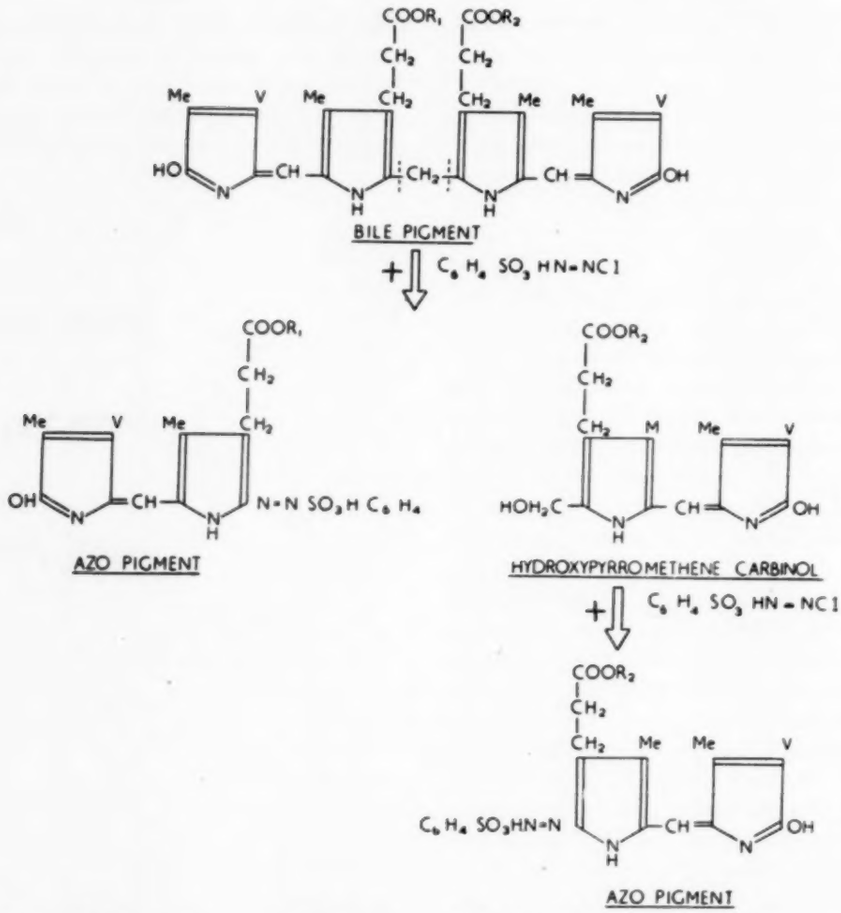

FIG. 2. The diazo reaction of bile pigments. (Bilirubin, R_1 and R_2 = H; pigment I, R_1 = glucuronyl, and R_2 = H; pigment II, R_1 and R_2 = glucuronyl.)

phoresis of partially purified dog bile he demonstrated that the direct pigment was constantly associated with glucuronic acid in a molar ratio of approximately 1:2. He has also partially purified the lead salt of bilirubin diglucuronide [*31*]. No infra red studies have been reported on this product but it is to be expected that they will indicate an ester structure. Confirmation that bilirubin is conjugated with glucuronic acid through its carboxyl groups has recently been given by Schachter [*32*], who has applied the observation that carboxyl glucuronides can be differentiated from other glucuronides by their capacity to react with hydroxylamine to give hydroxamic acids. He found that when urine from patients with jaundice was treated with hydroxylamine, appreciable amounts of hydroxamate were formed, the amounts being roughly proportional to the amount of direct reacting bilirubin converted to indirect bilirubin by treatment with 0.2 N NaOH for ten minutes at room temperature. (He also showed that pigment B, but not pigment A, reacts with hydroxylamine to give a hydroxamate of pigment A.) In this connection it is interesting to note that ester glucuronides of nicotinic acid [*33*] and aspartic and glutamic acids [*34*] have already been demonstrated in urine.

Pigment I has been separated chromatographically from other bile pigments and the molar ratio of pigment (expressed as bilirubin) to glucuronic acid has been found to be approximately one. This finding is consistent with the observation that pigment I yields both pigments A and B in the van den Bergh reaction, and favours a monoglucuronide structure for pigment I. The possibility that it is a complex of bilirubin and pigment II has, however, to be considered in view of our observation [*24*] that warming and evaporating a solution of pigment I sometimes resulted in its conversion to bilirubin and pigment II, when rechromatographed. We

have not been able to explain this and further information is needed to settle this point.

Pigment I gives a "direct" reaction in aqueous solution at an acid pH but the yield of azo pigments can be increased by the addition of alcohol. The reason for this can be deduced from the work of Overbeek and his colleagues [*29*] since the non-glucuronide, bilirubin-like, half of the pigment I molecule will be capable of forming both pigment A and hydroxypyrromethene carbinol; the latter will only form pigment A in the presence of alcohol and excess coupling agent. Knowledge of the amount of hydroxypyrromethene carbinol formed during the diazotisation of an aqueous solution of pigment I in serum is, therefore, necessary for a full understanding of the exact substances measured by the "direct" reaction. It seems probable that methods based on that of Malloy and Evelyn [*35*], which have proved so useful from a clinical standpoint, tend to give a direct reading which estimates pigment II and most of pigment I while the indirect reading determines bilirubin and some pigment I.

Indirect evidence supporting the glucuronide structure of pigments I and II has been obtained by determining the amount of alkali-labile glucuronides in icteric plasma [*27*]. It was found that increased amounts were present in plasma from patients with obstructive jaundice while normal levels occurred in plasma from infants with haemolytic disease, in which the chief bile pigment was bilirubin and not conjugated bilirubin [*36*]. It has also been shown that the ratio of direct pigments (expressed as bilirubin) to glucuronic acid in a large number of specimens of human fistula bile was approximately 2. Some scatter in the results was to be expected as substances other than bile pigments are known to be excreted as glucuronides. Also, varying amounts of pigment I (15 to 30 per cent) are to be found in bile in addition to pigment II [*37*].

Glucuronide conjugation of bilirubin has been demonstrated in the human, dog, rat and guinea pig. In view of the versatility of liver metabolism, the possibility that bilirubin may also be conjugated with other substances should be considered. At present no other mechanism has been proved. Sakamoto and his colleagues have, however, effected the partial purification of an ester-bilirubin from both dog bile [*38*] and urine from jaundiced subjects [*39*] which gives a direct reaction in the van den Bergh reaction but which does not contain glucuronic acid; the structure of this ester is not yet known. Also, we have observed that mild alkali treatment of human fistula bile does not result in complete conversion of the direct pigments to bilirubin; approximately 10 to 15 per cent of the direct pigment appears to be alkali stable [*27*]. Whether or not the presence of these alkali-stable pigments indicates the existence of non-glucuronide conjugates of bilirubin needs to be investigated.

The conjugation of lipid-soluble bilirubin with glucuronic acid results in the formation of a water-soluble pigment which will give a "direct" van den Bergh reaction. Bilirubin will also react "directly" if it is converted to its sodium salt or if solvents (e.g. ethanol), which are miscible in both water and chloroform, are used [*40*]. The essential factor appears to be the solubilisation of bilirubin in water. Jirsa, Večerek and Ledvina [*41*] showed that bilirubin could be made soluble by esterifying it with taurine. Both di- and monotaurobilirubin behave like the corresponding glucuronide derivatives in the van den Bergh reaction, chromatographically, in electrophoresis, and in ease of oxidation. The synthesis of a water-soluble bilirubin-sulphate which gives a direct van den Bergh reaction has also been reported [*42*]. None of these synthetic conjugated bilirubins has yet been found in bile.

Biosynthesis of Bilirubin Glucuronides. Van den Bergh's work showed that normally the liver converts bilirubin to direct bilirubin. The experiments of Yamaoka [*43*] on perfused rabbit livers provided evidence that the parenchymal cells are probably responsible for this conversion. Little is known of the mechanisms involved in the secretion of bile pigments by the liver cells. Hanzon [*44*] has concluded, from his studies in the rat, that secretion is localised in the mitochondria of the liver cells and that this involves passive penetration of the sinusoidal epithelium, followed by an active mechanism situated in the cells close to the two surfaces facing the sinusoids and the bile capillaries. This mechanism has a limited capacity since raising the concentration of the bilirubin in the plasma above a certain level will not increase the amount secreted in the bile. The maximal hepatic bile pigment clearance by normal rat livers has been estimated to be 61 μg./minute/100 gm. body weight ± Standard Deviation 8.0 [*45*]. Partial hepatectomy experiments have demonstrated that, in the adult rat, newly formed liver cells are as

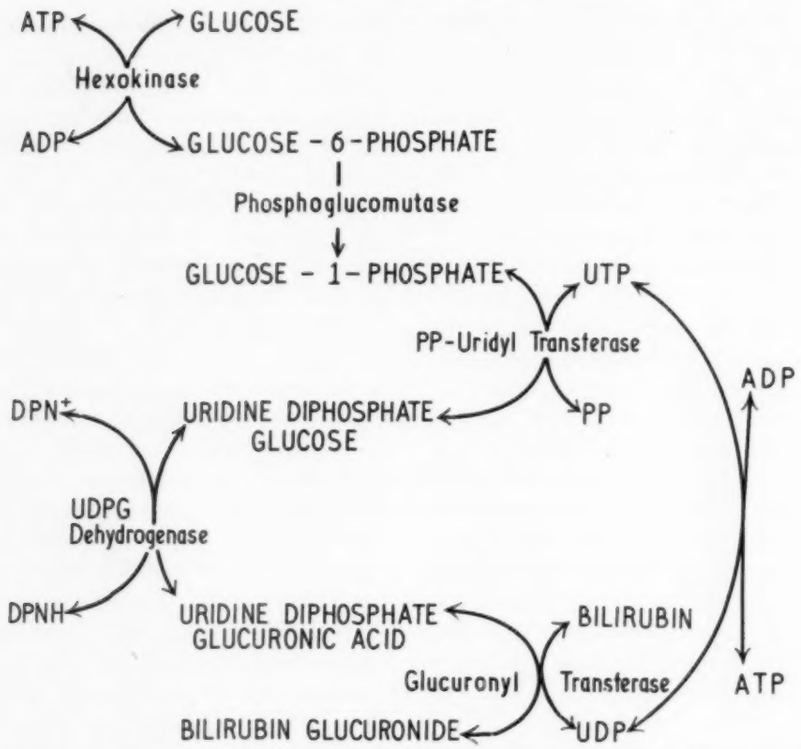

FIG. 3. A possible mechanism for the conjugation of bilirubin with glucuronic acid.

capable of extracting and secreting bile pigments as older cells.

The establishment of a glucuronide structure for direct bilirubin has provided the stimulus for an investigation of the enzymatic procedures involved in the production of these pigments. Almost simultaneously there have been reports by Schmid [*47*], Lathe and Walker [*46*] and Grodsky and Carbone [*40*] that liver tissue homogenates and slices will conjugate bilirubin in the presence of uridine diphosphate glucuronic acid, which acts as a glucuronyl donor in many glucuronide syntheses [*48*]. Conjugation has been estimated either by determining the increase in direct reacting pigments [*40,46*] or by the isolation of pigment B using paper chromatography [*47*]. The type of pigment synthesised has not been established. If it is the diglucuronide, which one would expect as this is the dominant pigment in rat bile, then it would be interesting to know if the monoglucuronide is an intermediate in its synthesis. Only crude enzyme preparations have been used and it remains to be determined how specific is the enzyme responsible for conjugating bilirubin. Lathe and Walker, and also Schmid, have found that conjugation is active in the microsome fraction of rat liver homogenates. A possible mechanism for the conjugation of bilirubin is given in Figure 3.

Extrahepatic Conjugation of Bilirubin. Bollman and Mann [*49*] studied the type of van den Bergh reaction in the plasma of dogs following hepatectomy. An initial indirect reaction was observed but as the amount of pigment increased the reaction became more rapid and bile pigments appeared in the urine. This suggested that conversion of bilirubin to a direct reacting pigment could take place in some organ other than the liver. Bollman [*50*] has recently reexamined these pigments using partition chromatography and found that the direct reacting pigment formed after twenty-four hours could be identified as pigment I; no pigment II was formed. Injection of haemoglobin into the hepatectomised dog increased the rate of accumulation of bilirubin and pigment I, but not of pigment II in the blood.

Weinbren and Billing [*51*] noted that when they saturated the hepatic secretory mechanism for bilirubin in the rat, by the intravenous infusion of bilirubin, pigment I but not pigment II accumulated in the blood, together with bili-

rubin. Bollman [*37*] has found that small amounts of pigment II do accumulate in the blood of hepatectomised rats, especially after bilirubin injections, but that the main pigments are bilirubin and pigment I. The existence of an extrahepatic mechanism for the conjugation of bilirubin to the monoglucuronide has, therefore, been established, but further work is necessary to ascertain whether or not the formation of the diglucuronide can, in some species, take place in organs other than the liver.

The site of this extrahepatic conjugation of bilirubin is uncertain. Grodsky and Carbone [*40*] have shown that rat kidney suspensions can conjugate bilirubin but at less than half the rate found with liver. Lathe [*52*] has confirmed this using slices. The nature of the glucuronide formed has not been established. Only slight activity was found in the brain and none in the muscle, spleen or blood [*40*]. It would be of interest to know if the kidney is normally involved in the formation of bilirubin monoglucuronide or if this is an emergency mechanism only employed in the absence of a functioning liver. Work is also needed to discover where pigment I is formed in patients with hepatitis or obstructive jaundice.

Nomenclature. The terms "indirect bilirubin" and "direct bilirubin" have no chemical or physiological significance. They will, therefore, be referred to as bilirubin and conjugated bilirubin, respectively, in the remaining discussion [*53*].

JAUNDICE

The yellow staining of the skin, associated with increased bile pigment concentrations in the plasma, is the result of either excessive production or inadequate removal of the pigment. Suitable technics for bile pigment analysis of tissues have yet to be devised but there is some evidence that bile pigments are bound to elastin [*3*]. This perhaps explains the fact that, in the jaundiced cadaver, large amounts of yellow pigment are seen in the skin, conjunctiva, mucous membranes of the intestines, the intima of the blood vessels, the kidney and the liver but not in the cornea, cartilagenous or nerve tissue. Humphrey, Neuberger and Perkins [*54*] have demonstrated that both tendon and skin contain significant quantities of plasma proteins, in addition to those present within the blood vessels, and Gitlin, Nakasato and Richardson [*55*] have obtained evidence that myoglobin of muscle is identical with serum albumin. It is possible that the staining of tissues may be caused by the presence of extravascular plasma albumin since both bilirubin and conjugated bilirubin are normally transported in the blood attached to plasma albumin [*14–16*].

A higher level of serum bile pigments is needed before jaundice is detected visually in infants with haemolytic disease than in adults with obstructive jaundice [*3*]. This suggests that the various bile pigments may be bound in a different way to the same or different tissue proteins, or that the water-soluble conjugated bilirubin can penetrate the tissues more easily than the lipid-soluble bilirubin. Further information regarding the binding of bile pigments to tissue proteins is needed.

Regurgitation Jaundice and Hepatitis. In regurgitation jaundice (whether due to obstruction or to liver damage) and hepatitis, bilirubin, pigment I and pigment II are found in the blood. (Fig. 4.) Quantitative studies of these pigments have been carried out by Billing [*56*], Bollman [*50*] and Baikie [*59*] using the technic described by Billing [*58*]. This chromatographic method is only approximately quantitative and there is a need for a new method of determining conjugated pigments which does not involve protein precipitation and which will be dependent on the different structures of the pigments. The relative proportions of the three pigments appear to be very variable; in a small series of cases Billing found 18 to 39 per cent as bilirubin, 38 to 62 per cent as pigment I and 15 to 35 per cent as pigment II. Bollman's results agreed with Billing's in showing that, except in cases of acute obstruction, pigment I is present in greater amounts than pigment II. This suggests the presence of a secondary metabolic insufficiency in the liver so that conjugation of bilirubin or conversion of the monoglucuronide to the diglucuronide is incomplete. In chronic cases it has been noted that the relative proportions of bilirubin and pigment I tend to increase as the jaundice continues.

Bollman observed that in hepatitis the proportions of pigments I and II varied at different stages in the disease in the same patient, but that in practically every instance greater amounts of pigment I were present. Higher concentrations of pigment II than pigment I were found in biliary obstruction due to malignancy or stone, if this was of short duration; similar findings were reported in the rat after ligation of

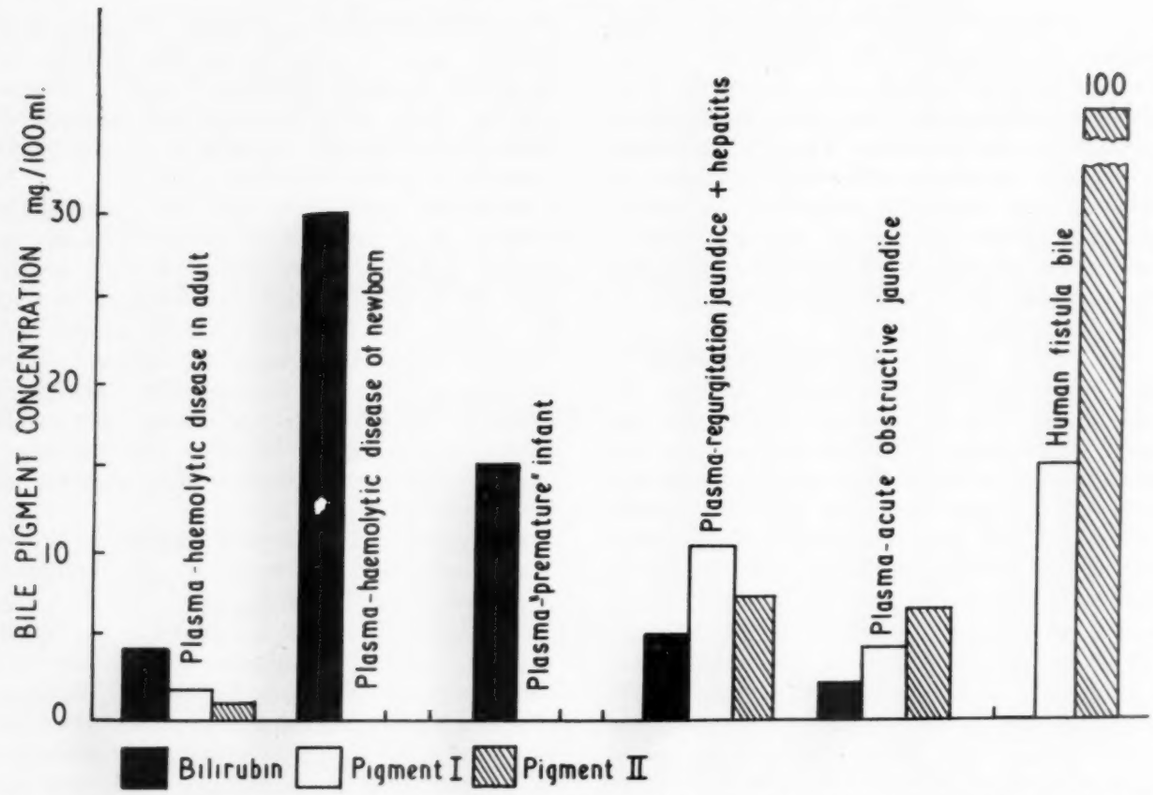

FIG. 4. Examples of the relative amounts of bilirubin, pigment I and pigment II found in plasma in various conditions, and in fistula bile.

the bile duct [*56*]. Knowledge of the relative proportions of pigments I and II may, therefore, be an aid in helping to decide between a diagnosis of obstruction or hepatitis.

In obstructive jaundice and hepatitis, both forms of conjugated bilirubin (but not bilirubin) appear in the urine. The relative proportions of these pigments has not been studied quantitatively but, as in icteric plasma, larger amounts of pigment I than pigment II appear to present. There seems to be no simple relation between the total amounts of conjugated bilirubin in plasma and in urine. In hepatitis, for example, "bilirubinuria" may be encountered in the initial stages of the disease with plasma levels as low as 2 to 3 mg./100 ml. plasma, while in the recovery period it will disappear as soon as the level has fallen to about 10 mg./100 ml. plasma [*3*]. No satisfactory explanation has been offered for this change in renal threshold and it might be worthwhile to investigate the possibility that different conjugated bile pigments are involved.

There is no reliable evidence to indicate whether the excretion of bile pigments is by glomerular filtration or by tubular excretion or by both. Histologic technics have demonstrated that in kidneys from icteric patients the glomeruli are colourless while the tubules are stained a deep yellow. In a dog with experimental jaundice Nizet and Barac [*59*] showed that the yellow staining was in the proximal convoluted tubules and the thick part of the loop of Henle. Renal clearance studies of both bilirubin monoglucuronide and bilirubin diglucuronide are urgently needed but are at present impossible owing to the lack of pure specimens of the pigments and suitable methods for their determination in urine.

Haemolytic Jaundice. In adults with haemolytic conditions bilirubin production, due to the breakdown of haemoglobin, may be increased as much as 5 to 15 times. It is theoretically possible that this may be the sole cause of the jaundice. But, because of the relatively enormous capacity of the adult liver to excrete bilirubin, this breakdown of haemoglobin must greatly exceed the normal daily 1 per cent if a substantial rise in plasma bile pigments is to be obtained. The rate of removal of intravenously injected bilirubin has been determined by

Weech, Vann and Grillo [*60*] in the human patient and by Weinbren and Billing [*45*] in the anaesthetised rat. From these studies it can be calculated that with a plasma bilirubin concentration of 10 mg./100 ml. plasma the normal liver could excrete the bilirubin equivalent of all the haemoglobin in the body in ten to twelve hours. The plasma level of bile pigments in haemolytic disease, therefore, usually only rises to 2 to 5 mg./100 ml. plasma.

The chief bile pigment in the serum of patients with haemolytic disease is bilirubin. (Fig. 4.) Small quantities of pigments I and II, which are responsible for the direct or biphasic reaction often encountered in the van den Bergh test, have been detected. Lack of a sensitive method at low concentrations of bile pigments has prevented their quantitative estimation. The presence of these conjugated pigments is suggestive of some liver derangement, which appears to be relatively specific, since alkaline phosphatase levels in the plasma are normal. The more frequent occurrence of jaundice in chronic extravascular haemolysis than in acute intravascular haemolysis could also be attributed to liver impairment.

Jaundice in the Newborn Infant. The dominant pigment in neonatal jaundice is bilirubin. (Fig. 4.) Unlike haemolytic jaundice in the adult, this is not necessarily associated with an overproduction of bilirubin. Mollison [*61*] has shown that, in the absence of haemolytic disease, the rate of breakdown of red blood cells in the newborn infant is not greatly elevated and a different explanation for the jaundice must therefore be sought. The absence of conjugated bilirubin in the plasma (Fig. 4) suggested to Billing, Cole and Lathe [*36*] that the accumulation of bilirubin in the plasma might be due to a metabolic block in the excretory process, at the stage at which bilirubin is converted to the conjugated pigment. They calculated that in many jaundiced infants the capacity of the liver to effect this transformation was reduced to 1 to 2 per cent of that in adults.

In 1957 Lathe and Walker [*46*] examined the livers of three newborn infants and found that their bilirubin conjugating capacity was very low, even in the presence of added glucuronyl donor (UDP glucuronic acid.) It appears that, like carbonic anhydrase [*62*], bilirubin glucuronyl transferase activity may be defective at birth.

Before birth the placenta and not the liver is responsible for the removal of bilirubin from the foetal blood. The infant is therefore not born jaundiced and only becomes so if the enzyme system responsible for the conjugation of bilirubin has not been fully developed. In small and premature infants, with immature livers, there is accordingly a greater tendency for plasma bilirubin levels to rise to considerable heights [*36*]. Although there is not yet any experimental proof, it is reasonable to assume that the characteristic fall in pigment concentration, which follows the rise, four to seven days after birth, is due to a corresponding rise in bilirubin transferase activity toward adult levels.

The result of this enzyme deficiency is that many newborn infants and in particular those suffering from haemolytic disease of the newborn (who have an additional increased pigment formation) are exposed to considerably higher plasma concentrations of bilirubin than are ever attained in the adult suffering from obstructive or haemolytic jaundice. Plasma concentrations of bilirubin may rise to 40 or 50 mg./100 ml. plasma, while in the adult, although similar total pigment concentrations might be attained, the bilirubin concentration would be less than 20 mg./100 ml. plasma.

Very occasionally, large amounts of conjugated bilirubin do accumulate in the plasma of newborn infants due to obstruction resulting from haemolytic disease ("inspissated bile syndrome"). These infants appear to have the ability to conjugate bilirubin so that dangerously high concentrations of bilirubin are not observed and exchange transfusion is therefore not indicated [*63*].

Congenital Hyperbilirubinemia. A rare familial condition, in which there is an abnormally high plasma bilirubin level in adults and children, has been described by Crigler and Najjar [*64*]. Schmid [*65*] has examined the bile of three such children and found that it is colourless and contains only traces of conjugated bilirubin. He found that the ability of these children to conjugate glucuronic acid with menthol, salicylic acid and metabolites of hydrocortisone was markedly depressed. These jaundiced individuals resemble a strain of homozygous jaundiced rats, described by Gunn [*66*], which have been examined by Schmid [*65*], Carbone and Grodsky [*67*], and Lathe and Walker [*46*]. They were found to be deficient in the same bilirubin glucuronyl transferase enzyme that is slow to develop in the newborn infant. Mild

cases of congenital hyperbilirubinemia are not uncommon and it remains to be seen if their reduced capacity to excrete bilirubin can also be explained on the basis of this enzyme deficiency.

Kernicterus. The very high levels of plasma bilirubin found in the premature infant, haemolytic disease of the newborn, familial hyperbilirubinemia and Gunn's rats may result in a pathological condition of the brain which, in the human, has been called kernicterus because of the deep pigmentation of the damaged areas. Claireaux, Cole and Lathe [*68*] have suggested that the primary cause of this brain damage is bilirubin. Evidence for the toxicity of bilirubin has been obtained from its inhibitory effect on the oxygen consumption of brain tissue [*69–71*], the lethal effect of large intravenous injections of bilirubin which produce brain pigmentation, and the demonstration of its uncoupling action on the oxidative phosphorylation of liver and brain mitochondria [*72*]. The action of bilirubin on the brain is probably related to the lipophylic nature of the pigment. This would explain the absence of kernicterus in the very jaundiced adult as the dominant plasma bile pigments are the water-soluble bilirubin glucuronides, which do not stain brain tissue.

ADDENDUM

In a recent communication, Arias and London [*73*] have demonstrated a deficiency of glucuronyl transferase activity in the livers of two adults with Gilbert's disease.

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Clinico-pathologic Conference

Hepatic and Renal Failure

STENOGRAPHIC reports, edited by Lillian Recant, M.D. and W. Stanley Hartroft, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

A THIRTY-THREE year old married white man entered the Barnes Hospital for the first time on July 7, 1956, with abdominal swelling of 10 months' duration. He died on August 31, 1956.

The patient began drinking in 1941 at the age of nineteen, consuming at least a half a pint of whiskey and several bottles of beer a day with additional "spree drinking" on weekends. His exact dietary intake during this period of time is unknown, but it is thought to have been inadequate to maintain satisfactory nutrition. In 1950 he passed an Army physical and served in Korea for two years. The patient dated the onset of his illness two to three years preceding this admission when he noted increased weakness and fatigability. Shortly thereafter he had the onset of numbness, tingling and cramping in his distal extremities. These symptoms were relieved by exercise.

In January 1955 he was hospitalized at the Veterans Administration Hospital in St. Louis. Positive physical findings included cheilosis, stomatitis, glossitis, a firm, non-tender liver palpable 6 fingerbreadths below the right costal margin, the spleen palpable 2 fingerbreadths below the left costal margin, liver palms and spider angiomas. Blood studies made on admission were within normal limits. The results of hepatic function tests were: bilirubin 2.5 mg./100 ml.; thymol turbidity, 11.1 units; prothrombin time, 73 per cent of normal; bromsulfalein, 46 per cent dye retained after twenty-five minutes; alkaline phosphatase, 9.4 Bodansky units; total protein, 8.1 gm./100 ml., albumin, 3.2; and globulin, 4.9. The serum cholesterol was 385 mg./100 ml. The upper gastrointestinal series was normal with no evidence of esophageal varices. Repeated urinalyses disclosed: trace to 3 plus proteinuria, 6 to 10 white blood cells per high power field, and a moderate growth of proteus organisms. He was treated with achromycin;® subsequent urine cultures were negative. He continued, however, to have proteinuria and "few" to "many" white blood cells per high power field. The total protein in one twenty-four-hour urine specimen was 1.27 gm. Addis count was reported as: casts, 0; red blood cells, 5,960,000 per cu. mm. and white blood cells, 752,000 per cu. mm. Blood urea nitrogen was 5.5 mg./100 ml. Phenosulfonthalein excretion test showed 17 per cent of the dye excreted in fifteen minutes in a urine volume of 15 cc. Intravenous pyelograms were reported to be within normal limits. During the eight weeks of this hospitalization, he was given a regular diet with high protein interval feedings and a multivitamin preparation. His weight increased from 119 to 128 pounds and his mouth lesions healed. Before discharge his laboratory tests included total protein of 7.9 gm./100 ml.; albumin, 3.8; globulin, 4.1; bilirubin, 0.7 mg./100 ml.; thymol turbidity, 4.5 units; alkaline phosphatase, 3.8 Bodansky units; and bromsulfalein, 24 per cent retention after forty-five minutes. Discharge diagnoses were Laennec's cirrhosis, cystitis due to proteus, vitamin B complex deficiency and chronic glomerulonephritis.

After discharge the patient resumed drinking. In October 1955 he noted abdominal swelling which was followed by progressive pedal edema. His ascites increased to the point where he could scarcely breathe lying down. It was for this reason that he sought medical attention. He denied jaundice, hematemesis and melena, but stated that he had had occasional putty colored stools and dark urine. There had been diminution of body hair for several months. He had no known exposure to hepatotoxins. For as long as he could remember, the patient had suffered from nocturia two to three times and occasional mild dysuria. There was no other urinary symptom until the development of ascites at

which time urgency and frequency appeared. He had not noted hematuria, pyuria or symptoms suggestive of renal stones.

The patient's father had had asthma and had died of a heart attack. His brother and one sister suffered from migraine. One sister had rheumatic heart disease.

Physical examination revealed the temperature of 37°c.; pulse, 120; respirations, 26; and blood pressure, 110/70 mm. Hg. The patient was poorly nourished. He appeared acutely and chronically ill. Fetor hepatitis was present. The skin turgor was poor. There were no spider angiomas. The tongue was reddened and papillae were diminished. Examination of the chest disclosed dullness and diminished breath sounds to the level of the fifth rib on the right posteriorly and to the seventh rib on the left. The cardiac rhythm was regular. The point of maximal impulse was in the midclavicular line in the fourth intercostal space. No murmurs were heard. Markedly distended veins were present over the abdomen. Drainage was away from the umbilicus. The abdomen itself was tense. A fluid wave was present. No organs or masses could be discerned. Bilateral pedal edema was present, greater on the right. There was a moderate, fine tremor of the hands. The deep tendon reflexes were active and equal. Unsustained ankle clonus was present. The position sense of the lower extremities was diminished.

The laboratory examinations were as follows: Hemoglobin was 8.1 gm./100 ml., and the white blood cell count, 14,900 per cu. mm.; differential leukocytic pattern: 1 per cent eosinophils, 1 per cent bands, 81 per cent polymorphonuclear leucocytes, 14 per cent lymphocytes, 3 per cent monocytes. The red blood cells were described as showing anisocytosis and poikilocytosis with slight hypochromia. Four urinalyses during the first two weeks disclosed specific gravity of 1.012 to 1.013, pH 5.5, trace of proteinuria and no sugar. An occasional white blood cell was seen in the spun sediments. A fecal specimen obtained on admission was guaiac positive; however, multiple subsequent stools throughout hospitalization were negative. Blood cardiolipin test for syphilis was negative. Blood chemical analyses; non-protein nitrogen, 49 mg./100 ml.; fasting blood sugar, 85 mg./100 ml.; amylase, 123 units; total protein, 7.3 gm./100 ml.; albumin, 1.3; globulin, 6.0; cholesterol, 113 mg./100 ml.; alkaline phosphatase 11.7 Bodansky units; 36 per cent bromsulfalein retention after forty-five minutes, cephalin flocculation test, 3 plus; sodium, 128 mEq./L.; potassium, 5.6 mEq./L., carbon dioxide, 18.5 mEq./L., chloride, 102 mEq./L. The electrocardiogram disclosed a sinus tachycardia but was otherwise within normal limits. Circulation time was normal and venous pressure was 230 mm. of saline. Roentgenographic examination of the chest showed a left pleural effusion. Throughout the patient's hospital stay the hemoglobin varied between 8.1 and 9.6 gm. per cent on multiple examinations.

Once in the hospital, the patient was fed a 3,000 calorie, 2 gm. salt, 40 gm. protein diet. He was given a multivitamin preparation, choline, methionine and glutamic acid orally. He was allowed to have fluids as desired. His tachycardia persisted, usually ranging between 90 and 110 but occasionally rising as high as 130. He was troubled with frequent loose bowel movements but the stools never became very copious. His fluid and electrolyte balance was followed closely. Mercuhydrin® failed to provoke diuresis. Urinary sodium remained at less than 1 mEq./L. On the third hospital day, 3,750 ml. of cloudy, yellow, opalescent ascitic fluid was removed. Specific gravity was 1.010; the protein content was 1.4 gm./100 ml. The fluid was thought to be chylous as there was no sediment on standing or on centrifugation. The paracentesis added greatly to the comfort of the patient. On the fourth hospital day, 6,900 ml. of similar fluid was withdrawn. A moderate number of inflammatory cells were seen in the sediment but no cancer cells were found. The patient tolerated this procedure well. The next day his dietary sodium was further decreased. The third paracentesis was performed on the seventh hospital day with removal of 12,000 ml. of yellow cloudy fluid. Following this procedure weakness developed and the patient's sensorium became clouded. He was given 800 ml. of ⅙ molar sodium lactate after which there was clinical improvement and a temporary rise in his urinary sodium. At this time the liver could be palpated 5 cm. below the right costal margin. Despite continued sodium restriction the patient's fluid intake remained at 2,000 to 3,000 ml. per day. The urinary output varied from 75 to 325 ml. per day. During the next five days his weight rose from 101 to 111 pounds with a concomitant fall in his serum sodium from 126 to 108 mEq./L. His non-protein nitrogen rose from 55 to 113 mg./100 ml. during the same

period. Despite his electrolyte disturbances no symptoms appeared to be associated with his low serum sodium. He looked mildly dehydrated. His total serum protein was 6.1 gm./100 ml. By means of parenteral administration of hypertonic sodium chloride and sodium lactate the serum sodium was raised to 134 mEq./L. His urinary sodium output remained at less than 1 mEq./L.

He was then given a high carbohydrate diet but he failed to eat all the food offered him. Occasionally he could be induced to take 1,500 to 2,000 calories, but more often his total intake was considerably less than 1,000 calories. He continued to accumulate fluid. In a week's time his weight rose from 121 to 134 pounds while his serum sodium fell from 134 to 124 mEq./L. and his non-protein nitrogen rose to 155 mg./100 ml. Salt poor albumin administered intravenously did not prevent this weight gain. A marked increase in ascites necessitated removal of 7,000 ml. of fluid on the twenty-fourth hospital day. On that and the following day, the patient received 240 mEq. of sodium intravenously without effect on the serum sodium level. The serum calcium was 10.1 mg./100 ml. and phosphorus was 7.9 mg./100 ml. On the twenty-seventh hospital day a new electrolyte plan of therapy was instituted. Dietary sodium was limited to 200 mg. and the total daily fluid to 1,000 ml. Serum electrolyte values returned to normal and the non-protein nitrogen fell to 75 mg./100 ml. over a three-week period. The patient's weight remained fairly constant after an initial loss of 6 pounds. He complained frequently of thirst and spider angiomas were noted over the left thorax and arms. Throughout his hospital stay the patient had displayed varying degrees of emotional lability and alertness. During the last four weeks of his life he had fluctuating periods of lethargy, drowsiness, obtundity and disorientation. No flapping tremor was noted although the tremor noted on admission persisted. On the forty-second hospital day, because of dyspnea, 1,500 ml. of cloudy yellow fluid was removed from the right side of the chest; the fluid contained 4,100 white blood cells/ml. Specific gravity was 1.011. No organisms grew on culture. The patient was afebrile at the time. During the last few days of his life he seemed progressively more disoriented and lapsed into coma. The day before death a second thoracentesis was performed and 1,000 ml. of fluid was removed. It is reported that 5 to 10 white cells per high power field were present. The patient died quietly on the fifty-eighth hospital day.

CLINICAL DISCUSSION

DR. CARL V. MOORE: This patient was thirty-three years of age at the time of his death. In tracing the genesis of his disease, several factors appear noteworthy. The adequacy of his diet was unknown. He said that he had eggs or cereal with milk for breakfast, sandwiches for lunch, and beer before and after dinner. He did not like beef or salads, but enjoyed fresh fruit. Unfortunately, no other details about his dietary habits are recorded. There was no history suggestive of viral hepatitis. In 1954, weakness and anorexia developed, there was a weight loss and the patient had numbness, tingling and cramping in his hands and feet. He was admitted to the Veterans Administration Hospital on January 13, 1955 where he stayed for two months. The pertinent physical findings at the time of this admission were cheilosis, stomatitis, glossitis, spider angiomas, decrease in axillary hair, hepatomegaly and splenomegaly. No edema, hemorrhoids or varices were demonstrable. There was evidence of disturbed hepatic and renal function and/or urinary tract infection. Not recorded on the protocol is the statement by one house officer that he found red cell casts in the urinary sediment on one occasion. The patient improved with rest, a moderately high protein diet, vitamin supplements, and treatment of the urinary tract infection.

I should like to open the discussion by concentrating for a moment on the elevated serum bilirubin value. We have no breakdown as to how much was the direct and how much the indirect reacting fraction. We do not know whether he had bilirubin or urobilinogen in his urine, but presumably both were present. We have been in the habit of talking about the direct reacting bilirubin as sodium bilirubinate and the indirect reacting bilirubin as bilirubinglobin. Recent evidence, as many of you know, indicates that the pattern of bilirubin metabolism implied by this terminology is incorrect. Dr. Harrington, would you review for us the current concept of the identity of the bilirubin pigments?

DR. WILLIAM HARRINGTON: The concept which Dr. Moore mentioned has been extremely useful clinically whether or not it is biochemically correct. It has been considered that bilirubin circulates in the blood largely as an indirect reacting pigment presumably bound to

protein, and that the bilirubin is split from the protein in the liver, linked to cation and excreted as a direct reacting pigment. However, as far back as 1918 van den Bergh postulated that the direct reacting pigment was really a polar compound of bilirubin. It is known from more recent studies that the direct reacting pigment is quite water soluble, whereas the indirect reacting pigment is relatively insoluble. On the basis of this more recent knowledge Schmid* reinvestigated the clinical nature of the two pigments. He found that the direct reacting pigment obtained from urine, bile or serum of patients with obstructive jaundice, would on hydrolysis become indirect reacting pigment. He further found that on hydrolysis, two micromoles of glucuronic acid were liberated from each micromole of bilirubin and could be separated by chromatography. The glucuronic acid is conjugated on the carboxyl groups of the bilirubin molecule.

Therefore, the present concept is quite different from the former one. It appears that (1) the indirect reacting pigment which requires alcohol for its detection in the usual van den Bergh determination is the relatively insoluble crystalline bilirubin; (2) the direct reacting pigment is bilirubin glucuronide; and (3) this conjugation with glucuronic acid takes place in the liver cell. Where the term sodium bilirubinate has been employed in the past, we should now specify either direct reacting bilirubin or bilirubin glucuronide. The indirect reacting pigment normally found in serum and found in increased amounts of hemolytic icterus is in fact crystalline bilirubin.

DR. MOORE: The diagnosis of Laennec's cirrhosis was made at the Veterans Administration Hospital. The evidence seems to be pretty clear-cut. There is nothing to suggest that he ever had viral hepatitis, or that he was ever exposed to hepatotoxins. It seems reasonable to believe that his hepatic disease was produced by the alcoholism and the associated poor diet. Dr. Shank, I wonder if you would review for us the present status of our knowledge about the importance of disturbed nutrition in the development of cirrhosis.

DR. ROBERT SHANK: The indications that deficiency of specific nutritional factors may be important in causation of cirrhosis go back a great many years. This disease was first called "alcoholic cirrhosis" with the implication that alcohol itself was the hepatotoxin. Although that remains a question, most people today accept the concept that alcohol is effective primarily by inducing poor diet. In addition, it was observed early that in cirrhosis there frequently occurred peripheral neuropathy with the clinical manifestations of beriberi, and so this observation too indicated that dietary deficiency was associated with the disease. More recently kwashiorkor, a nutritional disorder associated with fatty infiltration of the liver and presumably with later fibrosis has been described. This disease occurs in wide areas of the world, primarily in children and is due apparently to an inadequate intake of proteins after the time of weaning. Moreover, two types of experimental hepatic lesions may be produced in animals by dietary deficiencies. These are (1) the lesion of fatty liver which may be followed by the later occurrence of fibrosis (much like the lesion of portal cirrhosis in man) and (2) necrosis with actual widespread degeneration of the hepatic parenchymal cells. The deficiencies which appear to be important experimentally in inducing the first type of lesion are: choline, betaine and other materials which function as methyl group donors. If the diet of the experimental animal is deficient in these materials, fatty infiltration of the liver is likely to occur and if long persistent, fibrosis and cirrhosis may later evolve. In addition, vitamin B_{12}, folic acid and citrovorum factor are important in methyl group metabolism in man and deficiency of these vitamins may lead to fatty infiltration. With regard to the second lesion, other nutrients are responsible. Included are amino acids, particularly cystine, and vitamin E. Dr. Schwarz has described another material which he calls factor 3 which is important in protecting against dietary hepatic necrosis. He has isolated this factor from a number of sources but most particularly from Torula yeast.*

In what way do the experimental observations in animals contribute to our understanding of nutritional cirrhosis in man? It seems to me that we can conclude that cirrhosis is usually associated with fatty infiltration of the liver as an

* SCHMID, R. Direct reacting bilirubin, bilirubin glucuronide in serum, bile, and urine. *Science*, 124: 76, 1956.

* It has recently been announced by Schwarz that factor 3 is selenium. (Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.*, 79(12): 3292–3293, 1957.)

early lesion and is likely to occur when the diet is deficient in protein and certain essential amino acids. We do not yet know that the necrotic type lesion is ever induced by dietary deficiency in man.

DR. MOORE: Dr. Hartroft, do you have any comments you would like to make?

DR. W. STANLEY HARTROFT: It has often been stated that some of the dietary factors mentioned here are antagonistic in their action. For instance, cystine added to the diet is stated to enhance the fatty liver of choline deficiency, and it is true that at one time cystine was called antilipotropic for this reason. But the action of cystine is not explained quite this way. The diets that were used in such investigations were often deficient in sulfur-containing amino acids but this fact was not realized at the time. When cystine was added, it simply improved the dietary background and the animal ate more. It is well established that the more the animal eats of a choline deficient diet, the fatter his liver will become. Therefore although under the conditions cited, cystine did intensify the fatty liver, its action was in no way that of a specific antilipotropic agent. In the production of hepatic necrosis and fatty liver the greater the caloric intake, the greater the effect of the vitamin deficiency. Methionine deficiency should be included with cystine deficiency as a factor producing hepatic necrosis, because there must be an inadequacy of both sulfur-containing amino acids as well as alpha tocopherol and factor 3 in order to produce the experimental lesion.

DR. MOORE: We should now collect the rest of our data and ask Dr. Humphrey if he would be good enough to review the roentgenograms for us.

DR. WILLIAM HUMPHREY: In January 1955, routine chest examinations appeared normal. About one month later, the spleen was moderately enlarged and there were a few granular calcifications, presumably granuloma in the spleen. Gastrointestinal series showed abnormality of the gastric rugal pattern. The individual ruga appeared thickened and somewhat irregular in outline, evidence that I would interpret as indicating a gastritis. In addition, esophageal varices were present and were identified by their somewhat serrated margin in contrast to the perfectly smooth margin that we see in the esophageal mucosal folds. Intravenous pyelography performed at that time revealed the kidney to be within normal limits as to size with no abnormality of the calyxes or ureters. On admission to Barnes Hospital, roentgenograms of the chest showed displacement of the heart and trachea by a rather sizable collection of fluid in the right pleural space. Fractures of the right sixth and seventh rib with some callous formation were evident. At the final premortem examination of the chest, an increase in the collection of fluid in the right pleural space and a more recent fracture of the right ninth rib was seen. In summary, our diagnoses would be gastritis, splenomegaly, esophageal varices, right pleural effusion, oesteoporosis and pathological fractures of the right sixth, seventh and ninth rib.

DR. MOORE: At the Veterans Administration Hospital a diagnosis was made, not only of cirrhosis of the liver, but also of chronic glomerulonephritis. Coexistent renal involvement and hepatic disease have been described frequently. The hepatorenal syndrome occurs in acute yellow atrophy where renal involvement is primarily tubular. Tubular and glomerular changes have been described in experimental choline deficiency. Glomerular changes have been observed in dogs fed high protein diets and alcohol. Patek and his associates* reported that 7 per cent of 200 patients with cirrhosis had either acute or chronic glomerulonephritis and 11.7 per cent of sixty patients who came to autopsy had this diagnosis. The onset of the cirrhosis seemed to precede that of the glomerulonephritis. There was often evidence of hemolytic streptococcal infection preceding the onset of the acute nephritis or the exacerbation of chronic nephritis and the antistreptolysin titer tended to be elevated.

In our patient, red cell casts were reported by the house officer at the Veterans Administration Hospital as well as microscopic hematuria and persistent proteinuria. No hypertension was noted. The urine specific gravity was always low. Dr. Nickel, do you want to comment on the probable nature of the renal lesion. Can we differentiate between an exacerbation of chronic glomerulonephritis and pyelonephritis?

DR. JAMES NICKEL: In the nearly terminal stage of renal disease it is always difficult to decide between the infectious process and that

* PATEK, A. J., JR., SEEGAL, D. and BEVANS, M. The coexistence of cirrhosis of the liver and glomerulonephritis. Report of 14 cases. *Am. J. Med. Sci.*, 221: 71, 1951.

of chronic glomerulonephritis. I am influenced however by the visualization of red cell casts as well as persistent microscopic hematuria at the Veterans Administration Hospital. After subsequent admission to Barnes Hospital many centrifuged sediments were apparently examined and only white blood cells were seen. The course in this patient favors that of chronic pyelonephritis. The evidence in favor of that diagnosis includes the finding of persistent white blood cells in the sediment, at least one positive urine culture, and a small degree of proteinuria. The latter is much more consistent with pyelonephritis than with chronic glomerulonephritis. The absence of microscopic hematuria during his stay in this hospital further influences my decision. I would presume that the patient had an acute exacerbation of a chronic situation when he was first seen at the Veterans Hospital and continued chronic pyelonephritis when seen here.

DR. MOORE: Let us now quickly review the subsequent course. In March of 1955 following discharge from the Veterans Hospital, the patient promptly resumed drinking. In October, he noted abdominal swelling for the first time. The swelling progressed and he became bedfast. Nocturia, frequency and mild dysuria developed. On July 7, he was admitted to Barnes Hospital with an initial weight of 146 pounds. He was poorly nourished. He had fetor hepatitis, glossitis, a right pleural effusion, ascites, hepatomegaly, evidence of collateral venous drainage and edema. Laboratory data showed the expected evidences of impaired hepatic function. The last period of this patient's hospitalization was characterized by rapid accumulation of fluid, low urinary output, low urinary sodium, hyponatremia and rising non-protein nitrogen. Dr. Karl, will you discuss the pathologic physiology of these changes in fluid balance and electrolytes? Can we explain them solely on the basis of hepatic disease or do we have to postulate an additional renal component?

DR. MICHAEL KARL: This sequence of events is frequently seen in patients with advanced disease of the liver and need not necessarily be explained on the basis of an associated renal lesion. The pathogenesis is somewhat more complicated. Of all the factors playing a part in the formation of cirrhotic ascites, none is more controversial than the respective roles of sodium and water balance in relation to antidiuretic activity.

That patients with ascites retain sodium has been well established by Ralli, Eisenmenger, Relman and others. Relman states that renal blood flow and glomerular filtration may be normal, so that defects in these functions do not explain such sodium retention. Ralli, on the other hand, has shown that under certain circumstances, renal flow and glomerular filtration may be decreased. Bradley states likewise. The evidence that sodium retention is hormonal in origin is a little more convincing. For example, the sodium content of sweat, saliva and tears in cirrhotic patients is low, and such patients may also show increased tubular reabsorption of sodium. Furthermore, other hormones, notably estrogens, may cause salt and water retention in cirrhotic subjects. The aldosterone mechanism may also be an important factor in producing ascites.

Despite this sodium retention, it is not unusual to see patients with advanced cirrhosis present with extensive ascites, edema and hyponatremia without necessarily having had paracentesis or extensive mercurial diuresis. One explanation is that there may be a dilution of sodium mass by excessive water retention.

DR. MOORE: Is that why the venous pressure may be elevated?

DR. KARL: Venous pressure may be elevated in patients who have extreme water retention with increased plasma volumes. One explanation for water retention is related to the excess amounts of antidiuretic substance found in the urine of cirrhotic patients, as shown by Ralli. Stein, however, challenges these findings on the basis of methodology. If it is true that cirrhotic patients have increased antidiuretic activity, is this due to overproduction of antidiuretic hormones (ADH) or to the failure of the liver to inactivate these substances? Recently it has been shown that alcohol which in animals inhibits ADH, is ineffective in causing diuresis when given to cirrhotic subjects. Leiter has shown that pitressin® given to cirrhotic patients with ascites is inactivated at a normal rate. Neither of these explanations then, is acceptable.

Gabuzda* has outlined the changes that take place when patients of this type have paracentesis. He has observed that the hematocrit rises,

* GABUZDA, G. J., JR., TRAEGER, H. S. and DAVIDSON, C. S. Hepatic cirrhosis: effects of sodium chloride administration and restriction and of abdominal paracentesis on electrolyte and water balance. *J. Clin. Investigation*, 33: 780, 1954.

serum sodium values drop, peripheral edema tends to decrease, plasma volume drops, and ascites rapidly accumulates. The non-protein nitrogen may then rise as a result of the fall in circulatory plasma volume. It is his opinion that the sudden transudation of fluid from the plasma to the peritoneal cavity may in the same way invoke receptors that in turn cause increased antidiuretic activity.

DR. MOORE: Dr. Karl has pointed out quite beautifully the mechanisms involved in water retention, low urinary sodium output and low total urinary volume in hepatic disease alone. Dr. Bricker, do you think that all the electrolyte changes and impaired renal function can be explained solely on the basis of hepatic disease in this patient?

DR. NEAL BRICKER: There are at least two components to the abnormality that this patient presented. Initially, in the presence of ascitigenic cirrhosis, a moderate degree of azotemia developed with edema, ascites and hyponatremia. All of these I think are consistent with the physiologic abnormalities seen in cirrhosis. However, the events of the last two to three weeks of this patient's course do not fit into the usual sequence seen in the patient with cirrhosis prior to the terminal phase. The development of rapid and marked azotemia coupled with protracted oliguria suggest that something happened to this patient which may be called acute renal failure. The latter does not appear to meet the criteria for acute tubular necrosis and it may well fit into the ill defined category which some have called the hepatorenal syndrome. The fact that the blood urea nitrogen and phosphorus were extremely elevated is compatible with a very low glomerular filtration rate. The fact that this patient, however, was able to elaborate a urine virtually free of sodium to the last day of his hospital course implies a considerable degree of preservation of the tubular transport mechanism for sodium. That does not occur in acute tubular necrosis. However, it has occurred, in our experience, in patients with the oliguria associated with terminal cirrhosis. The final question of course is whether or not the patient had independent renal disease. There are some things that we cannot comfortably explain on the basis of cirrhosis; these include the red cells, the red cell casts and the pyuria. I think he certainly had some pyelonephritis. I do not think we have enough evidence either to rule in or out the possibility of glomerulonephritis.

DR. MOORE: It is impossible to include all the interesting possibilities in this discussion. However, one last question. Dr. Smith, do you think that the accumulation of ascites was so rapid in this man as to suggest that in addition to cirrhosis he probably had portal vein thrombosis? It's difficult for me to come to that conclusion because the spleen was not as large as one usually sees with portal vein thrombosis and he did not have much in the way of pain which would fit that occurrence.

DR. JOHN R. SMITH: I do not believe portal vein thrombosis needs be postulated, unless it occurred terminally. The accumulation of ascites in patients with cirrhosis of this type may be very rapid.

DR. MOORE: Do you think that the pleural fluid which develops on the right side of the chest in a person with massive ascites comes through the diaphragm?

DR. SMITH: No. The pleural fluid is a manifestation of extreme water retention within the circulatory space. Perhaps the decline of cardiac function, as part of nutritional failure in general induces pulmonary edema and surface transudation from the lungs. Possibly the size of the pulmonary lymphatics may determine the area of greatest pleural transudation; that is, if the capacity of the right pulmonary lymphatics were smaller than that of the left lung, greatest transudation of fluid from the visceral pleura of the right lung could produce a conspicuous pleural effusion, with less pleural fluid on the left side. Some patients with circulatory congestion have large left pleural effusions, possibly because the lymphatics of the left lung are less adequate, and more rapid transudation from that pleural surface occurs.

DR. ALBERT EISENSTEIN: When one is attempting to explain the sodium retention which occurs in cirrhosis, particularly when accompanied by ascites, the role played by aldosterone should not be overlooked. A low serum sodium in no way negates the importance of this hormone. One must also remember than when there is excessive secretion of aldosterone the intracellular concentration of sodium increases.

DR. MOORE: Yes, there are recent figures indicating that the urinary excretion of aldosterone by normal adults on a normal salt intake is less than 2 μg. per day, while patients with cirrhosis and ascites excrete as much as 18 to 24 μg. per day.*

* GORNALL, A. G., GWILLIAM, C. and HALL, A. E. D. Aldosterone exertion in various clinical studies. *J. Clin. Endocrinol. & Metab.*, 16: 950, 1956.

FIG. 1. Close-up of the cut surface of the liver reveals the bulging regeneration nodules of parenchyma (3 to 8 mm. diameter) surrounded by firm, depressed scar tissue.

I had wanted to discuss the anemia, the character of the ascitic fluid, etc., but we have to terminate the conference. This man had portal cirrhosis, probably on a nutritional basis. We have no good evidence to support a diagnosis of portal vein thrombosis. While chronic pyelonephritis or chronic glomerulonephritis may well have been present, the abnormal hepatic function in itself may well have accounted for the disturbance in water and salt metabolism. The gross findings will be presented by Dr. Kraus.

PATHOLOGIC DISCUSSION

DR. KRAUS: Grossly, subcutaneous venous channels over the thorax and abdomen of this emaciated man were prominently dilated. The marked wasting of soft tissues of the arms and thorax contrasted grotesquely with the massively distended abdomen from which 20 L. of turbid grey-brown ascitic fluid were recovered. The peritoneal surfaces were diffusely thickened by white fibrous tissue, and covered by loosely adherent coarse clumps and strands of fibrin. There was 1,000 cc. of clear fluid in the right pleural cavity; the left pleural cavity and pericardial cavity did not contain excessive fluid.

The liver was firm, small and contracted (1,150 gm.); the lobular architecture of its cut surface, which was tawny orange was replaced with rather uniform 3 to 8 mm. bulging nodules and was further distorted by dense bands of fibrous tissue connecting the portal areas and surrounding the nodules. (Fig. 1.) The portal vein and its tributaries were free of thrombi. The testes were small and atrophic (10 gm. each). Very small nodules of white fibrous tissue were present beneath the nipples.

Longitudinal, tortuous, dilated veins were present beneath the mucosa of the cardia of the esophagus. Superficial mucosal ulceration in this region was not associated with rupture or hemorrhage from these varices. The hemorrhoidal veins were numerous and dilated.

The spleen was small (180 gm.) and firm; its cut surface was brick red, with prominent fibrous trabeculae and numerous follicles.

The kidneys were slightly smaller than usual (120 gm. each). Their surfaces were finely granular and, on cut section, the cortices measured 8 mm. in width.

The lungs were atelectatic; however, the middle and lower lobes of the right lung which remained relatively expanded, were firm. The overlying pleura was thickened and covered by strands of organizing fibrous tissue. The cut surface was dry and granular so that fluid or purulent material could not be expressed from it.

Bacteria did not grow from cultures of either the heart's blood or ascitic fluid.

DR. HARTROFT: Microscopically, the hepatic architecture appeared extensively altered and distorted. (Fig. 2.) The parenchyma had been split into relatively small pseudolobules which were surrounded by rather broad scars. A few of the parenchymal nodules were much larger and contained radicles of the central veins as

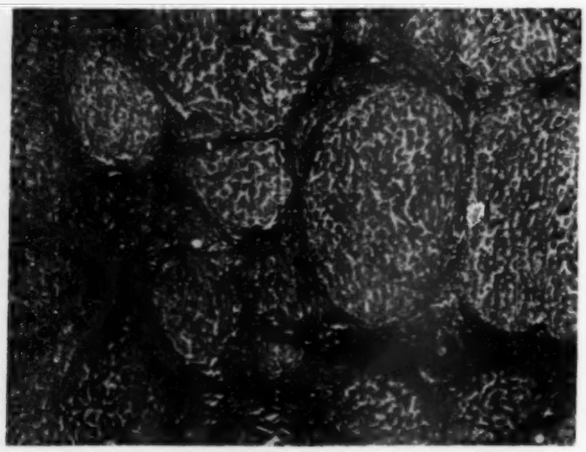


FIG. 2. A section of the liver shown in Figure 1 has been stained by Masson's method and photographed at low magnification with a Wratten B filter. Dense scars surround the two-cell thick plates making up the regeneration nodules of varying size.

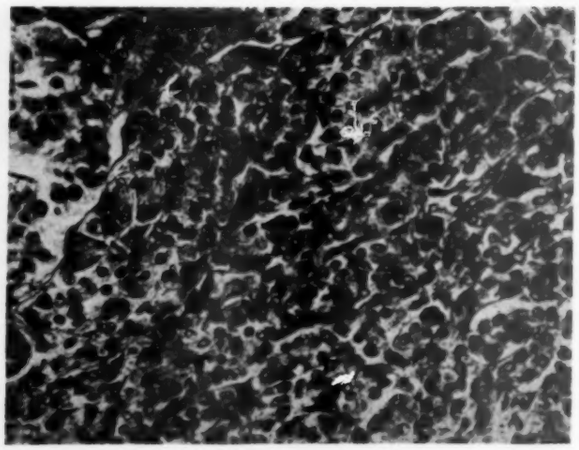


FIG. 3. Hematoxylin and eosin stain of a section of pancreas photographed at high magnification through Wratten B and G filters demonstrates the advanced degree of acinar tissue atrophy. Nuclei are separated by only small amounts of cytoplasm. The least atrophic exocrine cells lie near the islet partially included at upper left.

well as branches of the portal. In these regions particularly, evidence of hepatic cell regeneration was present in the form of two cell-thick plates as described by Elias. All the criteria (architectural distortion fibrosis and parenchymal regeneration) necessary for a diagnosis of cirrhosis were therefore present.

Abnormal accumulation of fat was not a prominent feature of these sections, but a few persistent fatty cysts were observed in the connective tissue bands thereby suggesting that at an earlier stage in the pathogenesis of the lesions, lipid had been present in abundant amounts.* Reticulin stains demonstrated the continuity of the collagen bands in fibrotic regions with the stroma of the surrounding parenchyma. In some of the liver cells at the edges of the scars, eosinophilic cytoplasmic clumps suggested Mallory's "alcoholic" hyaline.

The renal glomeruli exhibited a number of abnormalities, all of which were consistent with a diagnosis of late subacute or early chronic glomerulonephritis. Adhesions were present between the layers of Bowman's capsule and between adjacent lobules of many glomeruli. The basement membranes of nearly every glomerulus were thickened and many exhibited hyalinization of at least a portion of the capsule. Numbers of the afferent arterioles to the glomeruli had undergone degeneration and hyalinization so that the juxtaglomerular cells could not be clearly visualized. Crystalline casts (probably oxalate) were present in many of the tubules and in some instances in which tubular rupture had occurred, the casts were associated with a giant cell type of reaction.

Possibly the most astonishing microscopic change was found in the pancreas. (Fig. 3.) Nearly every acinus had undergone profound atrophy so that under the low powers of the microscope, the appearance was not unlike that of lymphoid tissue. The nuclei of the exocrine cells were surrounded by only small rims of cytoplasm, all the usual complex appearance of these cells (peripheral basophilia, zymogen granules etc.) having been lost. By way of contrast, the plump and relatively normal cells in the islets stood out very prominently.

Microsections of the lower lobes of the lung demonstrated the atelectasis observed grossly. In some regions the alveolar spaces were filled with fibrous tissue. Sections of adrenal contained abnormal quantities of lipid in the *zona glomerulosa* which appeared widened.

This case serves well to demonstrate the rather classical development of grave signs and symptoms of hepatic dysfunction in an alcoholic patient in whom an advanced degree of Laennec's cirrhosis had developed. Although atrophy of the exocrine portion of the pancreas is more commonly found in alcoholic subjects than in the general population [*1*], this association is perhaps not so widely recognized as the develop-

* STINSON, J. C., JR., BAGGENSTOSS, A. H. and MORLACK, C. G. Pancreatic lesions associated with cirrhosis of the liver. *Am. J. Clin. Path.*, 22: 117, 1952.

ment of damage to the liver. Not all authorities are in agreement concerning the question of whether or not glomerulonephritis is a sequela of cirrhosis or not, but there seems little doubt that the renal condition is encountered more frequently in those with cirrhosis (up to 10 per cent) than in the general autopsy population.* In any event, it is important to bear this point in mind when reviewing a case such as this one. Whether or not one agrees with the concept of a "hepatorenal" syndrome, there is abundant evidence from animal experiments that damage to these two organs (liver and kidney) can frequently be produced by a single toxic agent or dietary abnormality. Careful documentation of more cases such as the one presented may more clearly resolve this question in man.

Final anatomical diagnoses: Primary: (History of excessive alcoholic intake and poor diet, fourteen years); diffuse nodular cirrhosis of the liver; pancreatic acinar atrophy, advanced; testicular atrophy; gynecomastia, slight; spider-telangiectases of the skin; female distribution of pubic hair; absence of axillary hair; chronic passive congestion of the spleen; dilatation of esophageal veins, hemorrhoidal veins, and superficial epigastric veins; acute and chronic ulcerative esophagitis; subacute glomerulonephritis; emaciation; decubital ulcers; ulcers of the skin of the legs, bilateral with calcification; osteoporosis; ascites, 20 L. of turbid fibrinous fluid; marked thickening and fibrosis of all peritoneal surfaces; organizing peritonitis, marked; atelectasis and fibrosis of middle and lower lobes of right lung; revascularized thrombi of small pulmonary arteries; organizing purulent fibrinous pleuritis, right lung; crystalline casts in the proximal and distal renal tubules with focal tubular degeneration and granulomatous reaction; focal fibrous thickening of the endocardium, slight. Accessory: Arteriosclerosis of the coronary arteries, slight; arteriolar nephrosclerosis, slight; adhesion of the cusps of the aortic valve, slight; Lambl's excrescences of the aortic valve; chronic bronchitis; marginal emphysema in the upper lobe of the right lung; calcified nodule in the spleen and fibrous thickening of splenic capsule; Randall's plaque of a renal papilla; fibrous nodules in the diaphragm; hypocellularity of bone marrow.

Acknowledgment: Illustrations were prepared by Drs. F. Kraus and W. Stanley Hartroft of the Department of Pathology, Washington University School of Medicine.

* Patek, A. J., Seegal, D. and Bevans, M. The coexistence of cirrhosis of the liver and glomerulonephritis. Report of 14 cases. *Am. J. M. Sc.*, 221: 77, 1951.

Combined Staff Clinic

Polycythemia

THESE are stenotyped reports of Combined Staff Clinics of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Richard J. Cross.

DR. ALFRED P. FISHMAN: The term "polycythemia" is generally used as an abbreviation for "absolute polycythemia," i.e., an increase, to abnormal levels, in the total number of circulating red blood cells. The presence of polycythemia may be established by direct measurement of the volume of red blood cells in active circulation; in normal subjects at sea level this red cell "mass" is usually less than 30 ml. per kg.

Unfortunately, the methods for direct measurement of the red cell volume are still largely confined to the research laboratory; in practice, the presence of an absolute polycythemia is inferred from more indirect indices: the ratio of red cell volume to total blood volume as measured by the hematocrit, the number of red blood cells per cu. mm. of blood, and the concentration of hemoglobin per 100 ml. of blood. In general, the presence of a polycythemic state is suspected in males when the hematocrit exceeds 55 per cent, when the number of erythrocytes exceeds 6 million per cu. mm. and when the hemoglobin concentration exceeds 18 gm. per 100 ml. of blood.

At times, any or all of these indices may be misleading. For example, following chronic blood loss, hemoglobinization of red blood cells may lag behind red cell production so that the concentration of hemoglobin becomes a poor reflection of the total red cell mass. Similarly, in states such as burns, which are characterized by loss of interstitial fluid, the increase in hematocrit and number of red blood cells reflects shrinkage of the plasma volume, i.e., "relative polycythemia" rather than an absolute increase in the total number of circulating erythrocytes.

In most cases polycythemia is of the secondary type, which is usually distinguished from primary polycythemia on one of two general grounds: (1) the presence of a mechanism which can stimulate erythropoiesis, e.g., hypoxemia, or (2) the coexistence of certain clinical states, e.g., hypernephroma. More specific criteria for differentiation are summarized in Table I.

Systemic hypoxemia has been generally identified with secondary polycythemia; this association is emphasized by the first five clinical states listed in Table II. On the other hand, the last four states in the same table illustrate that hypoxemia is not an invariable antecedent of secondary polycythemia.

In the initial part of today's clinic, two different types of hypoxemic polycythemia will be considered: (1) the polycythemia of chronic exposure to reduced oxygen tensions, e.g., high altitude, and (2) the polycythemia of abnormal hemoglobin compounds, e.g., methemoglobin.

In residents at high altitudes, a linear relationship has been demonstrated between the degree, the duration and the continuity of the hypoxic stimulus on the one hand, and the level of polycythemia on the other [*1*]: the mean hemoglobin concentration of an average male resident at 15,000 feet exceeds that of his sea level counterpart by approximately 6 gm./100 ml. [*2*].

As may be seen in Figure 1, patients with congenital cyanotic heart disease also display a degree of polycythemia which is generally proportionate to the severity of the hypoxemia. By way of contrast, some patients with chronic lung disease and similar degrees of hypoxemia manifest only a slight polycythemic response; in such instances the limited polycythemic response is generally attributed to the inhibitory influence of chronic infection on erythropoiesis.

Many diverse types of evidence, such as biopsies of bone marrow, counts of reticulocytes in peripheral blood and studies of ferrikinetics, suggest an accelerated rate of formation of red blood cells at altitude; other indices, such as

chronic hyperbilirubinemia proportionate to the level of polycythemia, and abnormally high rates of excretion of urobilinogen in urine, indicate that this heightened erythropoietic activity is balanced by an augmented rate of destruction of red cells [*1,3*].

TABLE I
CRITERIA FOR DIFFERENTIATION BETWEEN PRIMARY AND SECONDARY POLYCYTHEMIA

	Polycythemia Vera	Secondary Polycythemia
Etiology	None demonstrable	Usually hypoxemia
Clubbed fingers	Absent	Often present
Splenomegaly	Generally present	Absent
Peripheral blood	Leukocytosis and thrombocytosis	Normal
Bone marrow	Generalized hyperplasia of all elements	Erythropoietic hyperplasia
Arterial blood oxyhemoglobin saturation	"Normal" or slightly decreased	Generally decreased in proportion to severity of polycythemia

TABLE II
SOME CLINICAL AND PHYSIOLOGICAL STATES ASSOCIATED WITH POLYCYTHEMIA

1. Residence at high altitude
2. Congenital cyanotic heart disease
3. Pulmonary arteriovenous fistula
4. Chronic pulmonary disease
5. Methemoglobinemia
6. Experimental neurogenic hypertension
7. Brain tumors and other lesions of the central nervous system
8. Cobalt administration
9. Miscellaneous tumors

Upon descent from altitude, human subjects undergo a decrease in their augmented red cell mass. This reduction involves two mechanisms: (1) a decrease in the rate of erythrogenesis, and (2) an increase in the destruction of red blood cells; of the two, the decrease in erythropoiesis is probably the more important [*1*].

Since an increase in circulating hemoglobin follows a sustained reduction in the oxygen supply to the tissues, it seems pertinent to evaluate what an increase in this oxygen-carrying capacity can contribute to satisfaction of the organism's requirements for oxygen. An understanding of respiratory physiology permits us to assess the effect of an isolated increase in oxygen capacity on the oxygen uptake in the lungs. It may readily be demonstrated that a major consequence of such an increase is an increment in the oxygen tension of mixed venous blood. However,

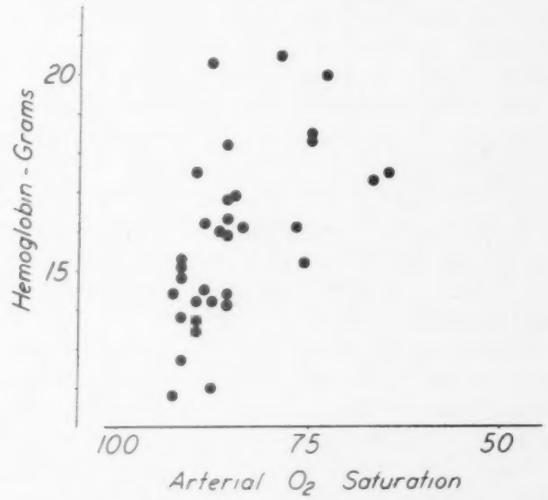


FIG. 1. Relationship of development of secondary polycythemia, as indicated by hemoglobin concentration of the blood, to hypoxemia (arterial blood oxygen saturation) in patients with congenital cyanotic heart disease.

this constitutes a detriment, rather than a gain, to oxygen uptake since, if uncompensated, it reduces the oxygen pressure difference across the alveolar membrane and diminishes the force responsible for driving oxygen into the pulmonary blood. Fortunately for the native resident at altitude, the increase in red cell mass is not an isolated phenomenon, and the increase in oxygen tension of mixed venous blood is accompanied by concomitant increments in both alveolar ventilation and in diffusing capacity [*1*].

In contradistinction to its ineffectiveness in promoting oxygen uptake in the lungs, the increase in hemoglobin mass does afford some physiologic gain to the tissues; thus, all else remaining equal, polycythemic blood effects higher oxygen tensions in the tissues than does normal or anemic blood.

Up to this point we have assessed the role of an increase in the *quantity* of circulating hemoglobin in promoting adequate oxygenation. It also seems pertinent to consider whether or not hypoxemic subjects undergo a change in the *quality* of the hemoglobin which affects either oxygen uptake or delivery. Some insight into

this problem may be gained from a study of the position of the oxyhemoglobin dissociation curves; for example, dislocation of the dissociation curve to the right of its usual position, under controlled conditions of pH, P_{CO_2} and temperature, indicates a diminished affinity of hemoglobin for oxygen; such a change in the hemoglobin molecule makes it possible for blood to be partially depleted of oxygen at an unusually small sacrifice in oxygen tension.

For a long while this favorable shift in the dissociation curve could not be demonstrated in residents native to high altitude; recently, with the advent of new analytic methods, evidence has been adduced that this decrease in affinity does occur [*1*]. This shift is in contrast to the normal position of the dissociation curve in polycythemia vera [*4*].

Unfortunately, little is known about the position of the dissociation curve in hypoxemic disease states. Evidence does exist that a shift of the curve to the right, similar to that described for residents at high altitude, occurs in patients with congenital cyanotic heart disease [*4*]; on the other hand, the situation in chronic lung disease remains to be defined.

This discussion has been concerned only with the gain afforded to the organism by the increment in the mass of circulating hemoglobin; we have not considered the untoward effects of polycythemia, i.e., abnormal viscosity of the blood, tendency to thrombosis, hypervolemia, and the problem inherent in many "homeostatic" mechanisms, i.e., the progressive increase in the physiologic cost of an ameliorating mechanism to a point beyond which it effects injury, rather than benefit, to the organism [*5*].

Many observers have called attention to the striking appearance of the native resident at altitude. This normal subject is generally a plethoric individual, with ruddy cheeks and cyanosis of the mucous membranes; his chest is overly large for his size and remains in a hyperinflated position throughout the respiratory cycle; his fingers are generally clubbed. Indeed, to the sea level clinician this normal resident is reminiscent of the patient with chronic obstructive pulmonary emphysema and right-sided heart failure. Of particular interest is the observation that the blood pressure in the pulmonary artery of the acclimatized subject at altitude is higher than in man at sea level, despite comparable levels of cardiac output [*1*]. This pulmonary hypertension finds clinical expression in dilatation and hypertrophy of the right side of the heart; such cardiomegaly occurs in almost half of the normal acclimatized subjects [*1*].

A final word about the native resident at altitude. Each visitor has noted with amazement the inordinate work tolerance exhibited by the individual born and raised at altitude. While the visitor pants and walks slowly, the resident carries heavy loads of ore and, on his "day off," engages in soccer football. However, it is also recognized that the unusual physiological adjustments which make such activity possible have been taxed to their utmost. Consequently, a minor respiratory infection, or impairment of pulmonary function following chronic exposure to dust in a mine, may aggravate the hypoxia to the point of complete incapacitation. Such respiratory complications may contribute to the chronic mountain sickness ("seroche") which afflicts some individuals born at altitude and disqualifies them for physical exertion there.

At altitude, one would be hard put to prove a diagnosis of polycythemia vera; by the same token, at sea level, one is generally reluctant to accept a diagnosis of polycythemia vera if there is an appreciable degree of systemic hypoxemia.

Systemic hypoxemia is generally established by analysis of arterial blood for oxyhemoglobin saturation; the latter is generally calculated, on the basis of analysis in a Van Slyke apparatus, as the ratio,

$$\frac{O_2 \text{ Content}—\text{Dissolved } O_2}{O_2 \text{ Capacity}—\text{Dissolved } O_2} \times 100.$$

By this method the hemoglobin in the arterial blood of normal subjects, as well as patients with polycythemia vera, may be expected to be 96 to 98 per cent saturated with oxygen. However, many patients with polycythemia vera have been reported to have "normal" saturations of 92 to 93 per cent. Although these values do represent mild hypoxemia, they are insufficient to account for the marked increment in red cell mass which characterizes polycythemia vera; they are similar to the inconsequential levels of polycythemia such as those which characterize normal subjects who reside approximately 5,000 feet above sea level.

Since the hypoxemia cannot account for the degree of polycythemia in polycythemia vera, it is of interest to consider whether or not poly-

cythemia vera may, in turn, effect arterial hypoxemia. Four mechanisms come to mind:

(1) *Analytic error:* This consists largely of the determination of oxygen capacity, wherein the ferric (non-oxygen transporting) form of hemoglobin may be converted to the ferrous form, thereby artificially increasing the oxygen capacity. This error is generally insignificant.

(2) *Diffusion limitation:* Impaired diffusion in polycythemia vera was described by Harrop [6] on the basis of experiments involving Krogh's single breath carbon monoxide method. This physiologic observation was interpreted as consistent with the well known tendency for sludging and thrombosis in polycythemia. However, the older methods for estimation of diffusing capacity were subject to considerable error and misinterpretation. Furthermore, theoretical considerations concerning rates of oxygen transfer, as well as recent observations by Drs. Turino and Brandfonbrener, using a steady state carbon monoxide method, indicate that diffusing capacity remains normal in polycythemia vera. Finally, in diseases characterized by impairment of diffusion there are usually overt manifestations of ventilatory insufficiency; these have not been observed in polycythemia vera.

(3) *Distribution defect resulting in perfusion of non-ventilated areas:* The possibility of systemic hypoxemia due to an inhomogeneity of alveolar ventilation and perfusion is suggested by anatomic evidence of increased capillarity, and by physiologic evidence of some alteration in distribution in other congestive states, such as mitral stenosis. However, various types of pulmonary function studies in patients with polycythemia vera have failed to demonstrate impaired distribution of inspired gas; furthermore, an appreciable abnormality in the distribution of mixed venous blood is inconsistent with the demonstration by Ratto, Briscoe, Morton and Comroe [7] that the hemoglobin in the arterial blood of most patients with polycythemia vera is generally 96 to 98 per cent saturated with oxygen.

(4) *Insufficient oxygen to saturate fully the large hemoglobin flow:* This mechanism probably does not operate in polycythemia vera for several reasons: (1) over short distances, corresponding to capillary diameters, diffusion may be expected to be so rapid as to constitute no time barrier to full oxygenation of the hemoglobin, (2) full saturation, while breathing ambient air at sea level, is also abetted by the large volume of oxygen returned to the pulmonary capillary in mixed venous blood, and (3) phlebotomy in polycythemic subjects with some degree of unsaturation fails to elicit appreciable changes in oxyhemoglobin saturation.

In brief, these considerations suggest that in polycythemia vera, mild arterial oxyhemoglobin unsaturation, previously designated as "normal," probably has its basis either in analytic error or in disease of the pulmonary parenchyma or vessels; it is apparently unrelated to the polycythemia *per se*.

Each series of patients with polycythemia vera includes one or more subjects with 70 to 80 per cent arterial oxyhemoglobin saturation. The basis for inclusion under the designation "vera" is the absence of *in vivo* or postmortem evidence of heart or lung disease. A mechanism which may account for the marked arterial oxyhemoglobin unsaturation in some of these patients was recently suggested by Newman, Feltman and Devlin [8]; it pictures, in sequence: primary polycythemia, vascular thrombosis with infarction of the respiratory center, alveolar hypoventilation, hypoxemia and finally, secondary polycythemia. In this evolution, secondary polycythemia emerges as a complication of polycythemia vera.

An alternate possibility is that the entire train of events is initiated by primary damage to the respiratory center. In this light, the polycythemia emerges as entirely secondary to alveolar hypoventilation and systemic hypoxemia. One such patient is well known to many of you since he has been repeatedly presented at conferences since 1953 [9]; a similar instance of injury to the respiratory center has been reported by Ratto et al. [7] from Dr. Comroe's laboratory in Philadelphia. These patients, with respiratory centers which are insensitive to normal stimuli, have normal lung volumes, normal maximum breathing capacity, no intrinsic lung disease and no defect in either distribution of inspired gas and mixed venous blood or in alveolar-capillary gas exchange. Despite these evidences of normal lungs and normal voluntary control of respiration, and in the face of augmented chemical stimuli, they manifest a persistent alveolar hypoventilation. During exercise, hypoxemia approaches precarious levels; the carbon dioxide tension increases and cyanosis becomes extreme, without evidence of respiratory discomfort or distress. The lack of response to the exercise stimulus, as well as to the carbon dioxide and pH

stimuli distinguishes the damage to the respiratory center from that observed in patients with chronic obstructive emphysema and carbon dioxide retention [*10*].

The laboratory demonstration of a depressed respiratory center is rather simple: the breath-

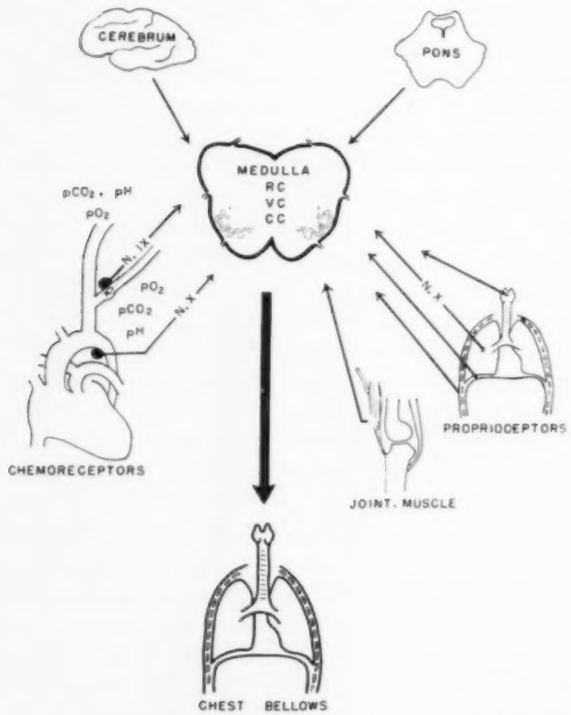


FIG. 2. Schematic representation of the mechanisms contributing to the normal ventilatory drive.

holding time is abnormally prolonged and the ventilatory responses to chemical and exercise stimuli are diminished. Much more complicated is the decision, once damage to the center has been established, as to whether or not the polycythemia preceded the damage to the center. This may be resolved only by careful documentation of the natural history of the disease in individual patients.

This group with primary damage to the respiratory center focuses attention on other primary mechanisms for alveolar hypoventilation. Some potential mechanisms may be deduced from Figure 2, in which are illustrated the elements contributing to the normal ventilatory drive. Abnormalities in any of these may elicit alveolar hypoventilation. For example, alveolar hypoventilation has been described by Auchincloss, Cook and Renzetti [*11*] and by others in obese subjects; Drs. Cameron and Turino have demonstrated to you in an earlier clinic that this is probably due to the augmented work of breathing associated with mobilization of the heavy panniculus. In a similar vein, recent observations by Bergofsky, Turino and associates in this laboratory indicate that alveolar hypoventilation has been a rather consistent characteristic of patients with various types of deformities of the chest, and that in patients with severe distortion of the chest bellows, polycythemia may occur. In these, as in the obese patients, the alveolar hypoventilation emerges as an unfavorable effect on total economy of the unconscious effort to minimize the work of breathing [*12*].

In concluding this section it seems pertinent to emphasize that the heightened awareness of primary alveolar hypoventilation elicited by primary depression of the respiratory center, or by an impaired ventilatory drive, or by impaired performance of the chest bellows, may well reduce the number of patients who would otherwise be recorded as examples of "primary polycythemia and systemic hypoxemia."

Up to this point the discussion has been largely confined to the polycythemia which arises from a deficiency in the amount of oxygen which is available to enter arterial blood; a final word may be said about the polycythemia which arises from an inability of hemoglobin to accept oxygen for transport, e.g., the polycythemia of methemoglobinemia.

Methemoglobin is an oxidation product of hemoglobin; as such, it cannot combine with oxygen and cannot function as an oxygen carrier. Although its untoward physiological effects are similar to those of other hemoglobin compounds, e.g., carboxyhemoglobin and sulfhemoglobin, it is distinctive in the ferric, rather than the ferrous, state of its iron.

The level of methemoglobin in blood depends on a balance between the rate of intraerythrocytic formation and destruction. Exposure to a wide variety of oxidizing agents, e.g., ferricyanide, nitrate and methylene blue, overwhelms the reducing system so that appreciable methemoglobinemia occurs. A congenital deficiency in an erythrocytic co-enzyme factor is probably responsible for congenital methemoglobinemia [*13*].

The impairment to oxygen delivery in acquired methemoglobinemia has two bases: (1) a decrease in the amount of hemoglobin available for oxygen transport, and (2) a change

in the shape and position of the oxyhemoglobin dissociation curve of the remaining hemoglobin, so as to impede the unloading of oxygen. Carbon monoxide and methemoglobin exert similar effects on the residual hemoglobin; this effect is illustrated in Figure 3, modified after Roughton and Darling [*14*]. It is this change in the dissociation curve which may account for the inordinate toxicity which attends removal of hemoglobin by oxidation to methemoglobin or combination with carbon monoxide, as compared with loss by bleeding [*15*].

A considerable degree of methemoglobinemia, i.e., a concentration of methemoglobin greater than 40 per cent of the blood pigments, is necessary before oxygen supply to the tissues by the remaining hemoglobin is appreciably compromised; minimum lethal levels are approximately 80 to 85 per cent. At such levels the remaining hemoglobin carries insufficient oxygen to meet the metabolic requirements of the resting organism [*16*]. Cardiovascular and ventilatory stimulation are generally absent until levels of 40 to 50 per cent methemoglobin are exceeded. This lack of respiratory stimulation contrasts sharply with the hyperventilation of altitude and stems from several factors: (1) arterial blood oxygen tension rather than content constitutes the ventilatory stimulus, (2) arterial blood oxygen tension remains normal in methemoglobinemia since the non-oxidized hemoglobin is fully oxygenated in the lungs, and (3) the lower portion of the dissociation curve, not required by normal subjects for usual activity, is called into play as an oxygen reserve for the tissues when hemoglobin levels are critically reduced. An unfortunate by-product of this lack of stimulation is an unheralded collapse which may occur if there is insufficient normal hemoglobin to supply the tissues with the oxygen necessary for the performance of vital functions.

The clinical manifestations and principles of treatment are fully detailed elsewhere [*17,18*]. Several diagnostic features are related to the physiological disturbances previously described. Thus, cyanosis in a patient without heart or lung disease, and without evidence of respiratory or circulatory distress, suggests the presence of an abnormal hemoglobin compound, such as methemoglobin or sulfhemoglobin. This impression may be supported by sampling brown-tinged arterial blood; the venous blood will remain muddy despite prolonged exposure to ambient air. Analysis of arterial blood will reveal that despite a high hematocrit, the oxygen capacity is unduly low; the oxygen content is correspondingly decreased, leaving the per cent oxyhemoglobin saturation and the tension of oxygen in arterial blood within normal limits. Finally, the diagnosis is established by spectrophotometric demonstration of the abnormal pigment.

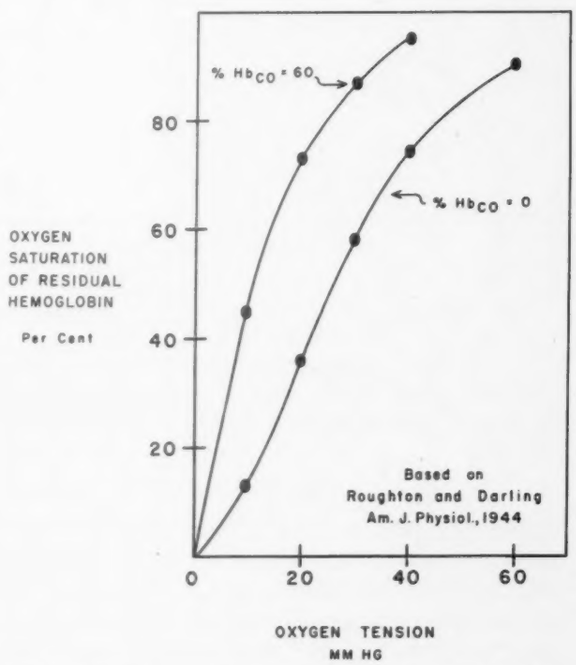


FIG. 3. The influence of carboxyhemoglobin on the shape and position of the oxyhemoglobin dissociation curve of the remaining hemoglobin. Modified after Roughton and Darling [*14*].

With respect to treatment, it is clear that unless concentrations of methemoglobin exceed 50 per cent there is no peril for the patient; the use of methylene blue and ascorbic acid in such a situation is consequently directed toward relief of symptoms such as headache and fatigue rather than to ward off circulatory or respiratory collapse.

It will have become clear to you, in the course of this presentation on polycythemia, that emphasis on blood itself has been sacrificed for the sake of its role in oxygen transport. In many respects, this is in accord with the dictum of the old sage, "Each man speaks most of what he knows best: the farmer of the plow, the sailor of the wind," etc. Our next speaker, Dr. Helen Ranney will bring us back to hematology and discuss polycythemia vera.

DR. HELEN M. RANNEY: In polycythemia vera, as in secondary polycythemia, an increased red blood cell mass is usually reflected in an increased concentration of hemoglobin, and an increased erythrocyte count and hematocrit. The frequent concomitant leukocytosis and thrombocytosis, and the clinical relationships of polycythemia vera, chronic myeloid leukemia and myeloid metaplasia have supported the classification of these disorders as myeloproliferative diseases. Mere classification, however, does little to clarify the pathogenesis of any of these diseases, and we shall devote the next portion of the discussion to some of the studies of polycythemia vera. We have somewhat arbitrarily chosen a few topics from the numerous aspects of polycythemia vera which have been investigated.

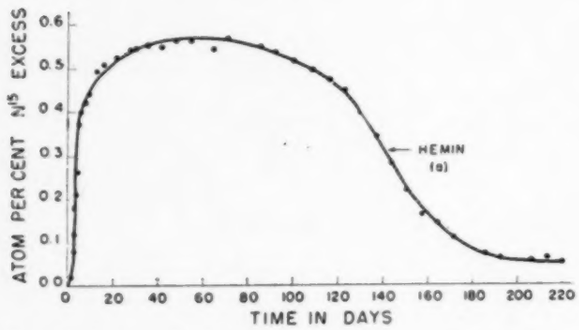
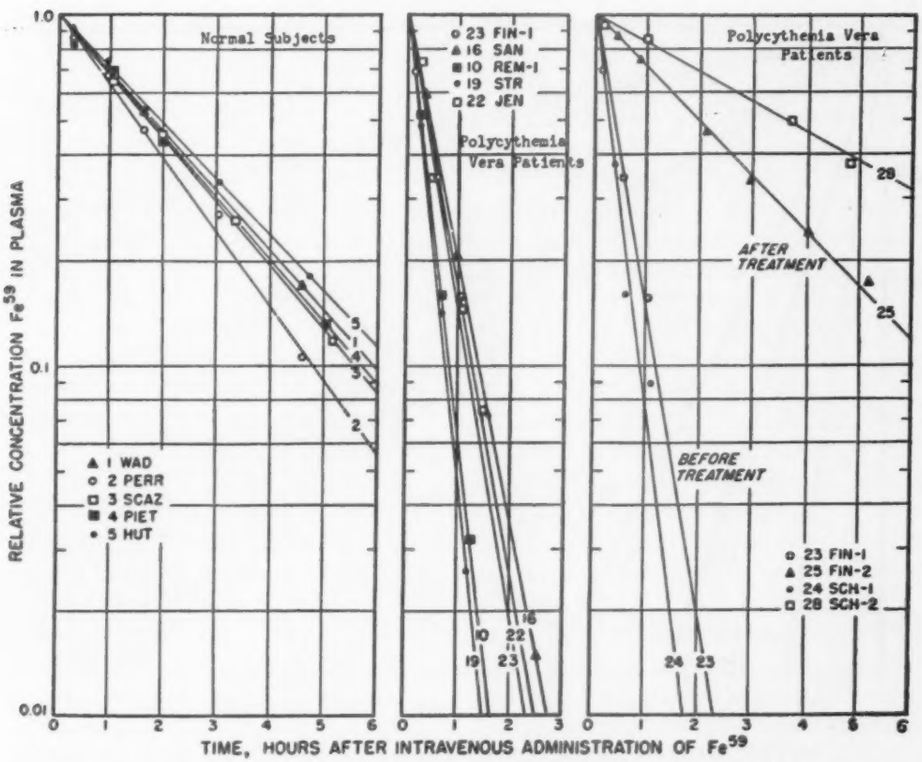


FIG. 4. Study of life span of erythrocytes of a patient with polycythemia vera. (From: LONDON, I. M., SHEMIN, D., WEST, R. and RITTENBERG, D. *J. Biol. Chem.*, 179: 470, 1949 [*21*].)

Leukocytosis commonly accompanies polycythemia vera, and many patients with polycythemia vera have terminal hematological findings which closely resemble those of chronic myeloid leukemia. It is of some interest, therefore, that there are certain metabolic differences between the morphologically similar leukocytes of polycythemia vera with a leukemoid blood picture and those of chronic myeloid leukemia. Valentine [*19*] has studied the alkaline phosphatase of the leukocytes of these two diseases: in chronic myeloid leukemia, the unit alkaline phosphatase is low; in polycythemia vera and other myeloproliferative disorders the leukocyte alkaline phosphatase activity is high. A similar contrast in leukocyte glycogen content is found between the morphologically similar leukocytes of chronic myeloid leukemia and polycythemia vera with a leukemoid reaction. Low glycogen values are found in chronic myelogenous leukemia, high values in polycythemia vera with leukemoid features [*19*]. The fact that such metabolic differences may be observed in morphologically similar leukocytes in these disorders is of considerable interest; its meaning in terms of etiology or interrelationships of these diseases is unknown.

The second topic to be mentioned, that of blood volume in polycythemia vera, is scarcely a new one, but with the introduction of radioactive chromium for labelling erythrocytes, accurate red cell volume measurements have been facilitated. Various studies have indicated that polycythemia vera and secondary polycythemia are accompanied by an increased red cell mass and a normal or low plasma volume [*20*]. Because of the overlap in range of hemoglobin concentration of subjects with high normal values and patients with polycythemia vera, direct measurement of red blood cell mass using P^{32} or chromium may occasionally add useful data in the diagnosis of polycythemia vera. The maximal diagnostic usefulness of the direct measurement of red cell volume would appear to be in patients with hematocrit values of 50 to 56 per cent; it is in this group that the diagnosis of polycythemia may be difficult to establish.

The observation that polycythemia vera results from an overproduction of erythrocytes rather than from a prolonged erythrocyte life span was made by London, Shemin, West and Rittenberg [*21*], who studied a patient with typical polycythemia vera. (Fig. 4.) When erythrocytes were labelled after feeding N^{15}-glycine, and the concentration in the heme determined at intervals during the four-month observation period, no evidence for an abnormality in life span of the erythrocytes was obtained. The rate of heme production exceeded the normal state. Subsequently, studies of Lawrence and co-workers [*22*], who employed C^{14} as an isotopic label and determined the C^{14} concentration in hemoglobin, were consistent with the presence of two populations of erythrocytes in patients with polycythemia vera; one population with a normal life span and another with markedly diminished survival time. However, studies of erythrocyte survival in other patients with polycythemia have confirmed the observations of London et al. [*21*], and for the usual patient with this disease the generally accepted conclusion is that the erythremia results from over-



$$\text{Relative Concentration} = \frac{\text{Concentration at any } t}{\text{Concentration at } t = 0}$$

FIG. 5. Plasma iron disappearance curves in normal and polycythemic patients. HUFF, R. L., HENNESSY, T. G., AUSTIN, R. E., GARCIA, J. F., ROBERTS, B. M. and LAWRENCE, J. H. *J. Clin. Investigation*, 29: 1045, 1950 [*23*].

production of red blood cells which survive for the normal period of four months.

A third aspect of polycythemia vera which has been studied extensively is that of iron metabolism in this disease. Most of the ferrokinetic studies are based upon data obtained following the intravenous injection of Fe^{59}, bound to the transferrin of fresh plasma or of Cohn fraction IV-7. The initial observations and calculations for this type of study were made by Huff and his associates in 1950 [*23*]. (Fig. 5.) From the rate of disappearance of injected Fe^{59} from the plasma, the plasma iron turnover, in milligrams per day or in milligrams per 100 cc. of whole blood, may be calculated. Huff originally also devised calculations of red cell formation based upon the amount of radioactive iron appearing in the erythrocytes and the plasma iron turnover rate. However, for estimations of red blood cell formation from such data one must make certain assumptions about incorporation of plasma iron in red blood cells, and about erythrocyte survival; these assumptions may not be true in abnormal states. Consequently, we shall confine this portion of the discussion to studies of plasma iron disappearance rates in polycythemia vera.

If the clearance rate of iron from the plasma is determined over the course of a few hours, it may be expressed as a single exponential; however, if the observation period is prolonged the clearance rate becomes slower, implying equilibration with one or more extravascular iron pools.

In Figure 5, from the studies of Huff and collaborators [*23*], are shown the Fe^{59} disappearance curves in normal subjects and in patients with polycythemia vera. Accelerated Fe^{59} disappearance rates are found in iron deficiency as well as in states of increased erythropoiesis. Whether or not increased erythropoiesis and related depletion of iron stores provide the full explanation of accelerated plasma iron disappearance rates seen in polycythemia vera is not known. Lawrence and co-workers have interpreted these data in support of their finding of two populations of erythrocytes; the increase in erythropoiesis necessary to maintain an eryth-

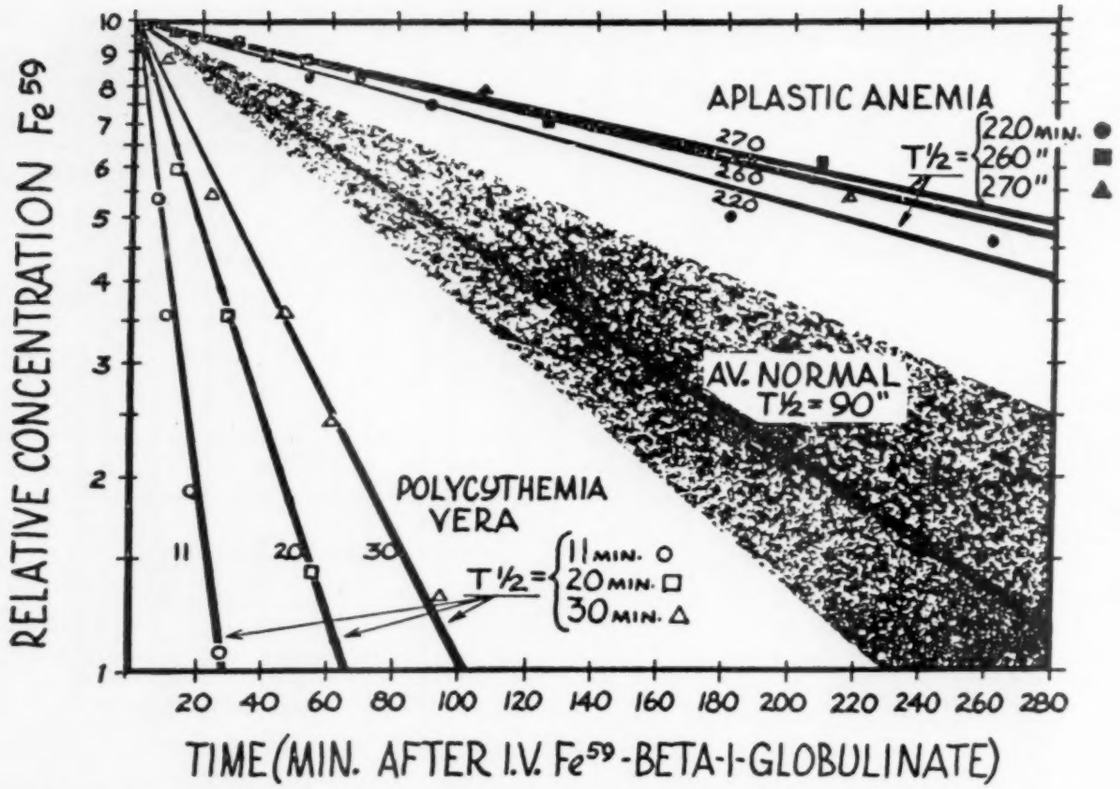


FIG. 6. Plasma iron disappearance curves. WASSERMAN, L. R., RASHKOFF, I. A., LEAVITT, D., MAYER, J. and PORT, S. *J. Clin. Investigation*, 31: 35, 1952 [*24*].

rocyte population of short life span would result in a more rapid turnover rate for iron. It is evident from Figure 5 that, following treatment of polycythemia with P^{32}, the plasma iron disappearance rate returns to normal. Similar findings have been published by Wasserman et al. [*24*]. (Fig. 6.) Again, here are shown the markedly accelerated disappearance rates from the plasma of Fe^{59} in polycythemic patients. Some acceleration of plasma iron disappearance rate may be encountered in secondary polycythemia, but the deviation from normal is not as great as in polycythemia vera. Determination of plasma iron disappearance rate has been used in some clinics for the diagnosis of polycythemia vera and for evaluation of the need for re-treatment, particularly with P^{32}. Generally, one does not need such a technic for these purposes, since observation will usually yield both diagnosis and an estimate of the need for re-treatment.

An intriguing observation on the blood from patients with polycythemia vera has recently been made by Riley and associates at Sloan-Kettering Institute for Cancer Research [*25*]. This observation concerns differences in whole blood chromatograms of patients with polycythemia vera, normal subjects and patients with a variety of other diseases. In approximately 90 per cent of patients with active polycythemia vera, abnormal patterns consisting of a bizonal distribution of erythrocytes were obtained. This bizonal pattern has been shown to be independent of the plasma and of the hematocrit. In contrast, 90 per cent of patients with secondary polycythemia have completely normal patterns. As Riley points out, "if the chromatographic segregation of erythrocytes into two populations in this disease represents a valid distinction, in contrast to a possible unknown artifactual effect, this may provide an important means of studying erythrocyte alteration not detectable by microscopic or other procedures" [*25*]. The chromatographic findings would not be inconsistent with the observations that the red blood cells of at least most patients with polycythemia vera survive normally. In other situations, for example hereditary spherocytosis after splenectomy or sickle cell trait, normal survival time of abnormal erythrocytes has been demonstrated.

DR. FISHMAN: In the two previous presenta-

tions, the problem of the stimulus to erythropoiesis was deliberately side-stepped. As you all know, definition of this stimulus has recently been attempted by a variety of ingenious investigations; the results are difficult to assess by the neophyte who is not thoroughly familiar with the problems that attend such types of investigation. Dr. Joseph Turner will provide a critical assessment of the present status of "the stimulus to erythropoiesis."

DR. JOSEPH C. TURNER: During the past several years much attention has been given to the hypothesis that erythropoiesis is regulated by humoral substances. This subject is important enough to demand most of the time allotted to me, but we must first round out the description of polycythemias with brief mention of two interesting varieties, one of them natural, and the other experimental, and both peculiarly relevant to the topic of control of red cell production by hormones.

First, we note the occasional association of polycythemia with tumors whose origin may perhaps be almost any tissue (e.g., hypernephroma, uterine fibroids) but most often with hemangioblastoma, particularly of the cerebellum [*26*]. The coincidence of two unusual diseases does itself suggest some specific relationship; moreover, successful extirpation of the tumor has been followed by disappearance of the erythremia. It may therefore be conjectured that the neoplasms elaborate some substance which stimulates red cell formation.

Secondly, there is cobalt polycythemia, which can be induced in a variety of animals by oral or parenteral administration of the metal, usually in the form of its chloride. In explanation of the effect it has been suggested that cobalt poisons enzymes of (nucleated) erythrocyte precursors in such a way as to produce anoxia, which would be expected to stimulate red cell production. However, it appears that cobalt does not affect the oxygen consumption of marrow even in concentrations much greater than those required to suppress heme synthesis [*27*]. One is tempted, once more, to account for the findings in terms of a humoral erythropoietic factor the activity of which is enhanced by cobalt.

That such a factor exists is not a new idea, but it attracted fresh interest with the demonstration that if one of a pair of parabiotic rats is made to breathe 10 per cent oxygen while the mate breathes air, the bone marrows of both animals reveal hyperplasia of erythroid elements [*28*]. This is taken to suggest that some humoral substance from the hypoxic rat stimulates blood formation in the animal breathing air. Clinical counterparts have been found in patients with the type of patent ductus arteriosus which arises distal to the subclavian artery and exhibits permanent reversal of blood flow. In the few such people in whom careful studies have been made, the sternal marrow, although receiving fully oxygenated arterial blood, has shown a hyperplasia like that which is seen in the marrow of the iliac crest, where increased erythropoiesis is expected because the arterial supply is markedly unsaturated. That is to say, the percentage of normoblasts in both places may be as much as twice the normal figure (e.g., 50 per cent rather than 20 to 30 per cent).

Morphological observations of this kind, although provocative, are hardly convincing demonstrations of an erythropoietic hormone, since, for one thing, there is some uncertainty as to whether or not the existing polycythemia might not itself cause morphologic changes in the marrow which could be interpreted as an apparent hyperplasia. Therefore, numerous attempts have been made within the past few years to bring to bear on the problem other, more dynamic kinds of evidence. There are already those who seem to be convinced that the hormone in question has been clearly demonstrated to exist, and, indeed, the word "erythropoietin" has been coined.

Some difficulty attaches to the methods of assay and their interpretation. Increased blood formation may be measured in terms of reticulocytosis, or increased rates of incorporation of iron into hemoglobin, or increased red cell mass. Must the hypothetical hormone produce all these changes? By no means, for the synthesis of a cell and its liberation into the blood is obviously so complex a process that there might well be a number of humoral factors that could affect one or another part of it. On the other hand, it is surely true that what one would like most to see is test material derived from blood plasma that will produce in the normal animal an increased output of normal red blood cells as determined by the development of polycythemia.

Several methods of assay for "erythropoietin" have been used. Native or treated plasma or serum from human beings or animals has been injected into rats or rabbits, and the effects on blood formation have been noted. Often the donors have been "stimulated" to form increased

amounts of erythropoietin by making them anemic through bleeding or with phenylhydrazine.

One series of experiments has been concerned with showing that rather large volumes of plasma or serum from anemic rabbits will upon intravenous administration to normal rabbits produce a rise of reticulocytes in the circulating blood of the recipients, whereas control serums from normal rabbits do not. Evidence is given that the essential material in the "anemic plasma" is a gamma globulin, while albumin and other fractions possess no activity [*29*]. In a report from another group of workers [*30*] increments of red cells and hemoglobin were produced in rats by parenteral injections of extracts of the plasma of anemic rabbits. Here the extracts were prepared after acidification and heat, which could denature much of the protein. It is worth noting that the test serums came from rabbits made anemic with phenylhydrazine. Other investigators who tried to repeat these experiments [*31*] found significant increments of hematocrit only in those rats that received extracts of plasma from rabbits given enough phenylhydrazine to produce obvious damage to the liver and spleen, as well as anemia. Where severe poisoning of the donor animals by phenylhydrazine is important for the "activation" of their plasmas it is clear that a toxin rather than a physiological hormone might be implicated. At the same time, attempts to demonstrate an erythropoietic factor in the plasma of rabbits made anemic by bleeding alone were unsuccessful. Still other experiments of a similar kind [*32*] have been reported to show in the recipient rats appreciable increases in numbers of red cells but no concomitant rise in hemoglobin or hematocrit. There is sufficient disagreement among these several reports to make interpretation of the findings rather difficult.

More recent work has employed yet another method of assay, to wit: Fe^{59} uptake by red cells in the rat [*33*]. Injections of plasma from anemic animals or of plasma from patients with various anemias appear to accelerate the rate of incorporation of the isotope. The effect can be shown most clearly in the acutely starved rat. The method has the attraction of being technically simple, and it is generally accepted that iron uptake is directly related to the rate of heme synthesis. On the other hand, the interpretation of metabolic data obtained under the condition of starvation is not always easy. It is possible to accept the data as indicating an increase in the rate of heme synthesis, and yet to be uncertain in how far this reflects the whole of erythropoiesis.

To sum up what has been said, various methods of assay have been applied to the problem of demonstrating the existence of humoral factors which stimulate erythropoiesis, and the results suggest that there may be one or more substances variously influencing one or another phase of the elaboration of the mature red cell. However, the significance of many of the reported findings will become established only after the test materials have been concentrated and purified.

Next, and finally, is the matter of control of erythropoiesis by the pituitary [*34*]. The feeding or injection of pituitary extracts has been shown to stimulate erythropoiesis in hypophysectomized animals, and the principal question at issue has been whether the effect is produced by a specific hormone with bone marrow as its target or by more familiar pituitary hormones through their effect on other end-organs and thus on metabolism. It has been particularly difficult to separate erythropoietic activity from ACTH activity but recent evidence indicates that this has been virtually accomplished, although the erythropoietic factor has not yet been isolated. No doubt the findings will be confirmed and extended one day to the point of assay of the hormone in man, and it will then be possible to appraise its relation to the polycythemias.

It should be clear that the hormonal control of red cell production appears at the present time to be far from simple. The experiments that have been reported involve substances that seem to differ widely both in their chemical nature and in their biological effects. As has so often happened, the differences may be reconciled in considerable part by improvements of method and refinement of the test materials. But they may not, and furthermore it would be unfortunate if this morning's discussion should leave the impression that we are close to an explanation of polycythemia in biochemical terms. The central fact remains: lowering of the oxygen tension of the environment stimulates hemoglobin formation throughout the animal kingdom, even in those primitive invertebrates whose blood pigment is not intracellular, but flows in solution in the plasma. Whatever may be the mechanism here, it is certain that in the higher organisms its operation is complicated by the appearance of

other influences, and we are now only at the beginning of a proper analysis of the problem.

DR. FISHMAN: Are there any questions or comments from the audience?

DR. GUSTAVE A. LAURENZI: Is there any difference in the clinical manifestations of acquired methemoglobinemia, as contrasted with congenital methemoglobinemia?

DR. FISHMAN: In general, patients with acquired methemoglobinemia manifest few symptoms, except for dyspnea on exertion, until levels greater than 30 to 40 per cent of methemoglobin are reached; greater concentrations are associated with fatigue, headache and severe dyspnea. Patients with congenital methemoglobinemia suffer even less clinical disability; one mechanism which may contribute to their well-being, in contrast to patients with acquired methemoglobinemia, is the normal position of the oxyhemoglobin dissociation curve [*35*].

DR. DANIEL LARSON: Would you care to comment on the mechanism by which methylene blue, a methemoglobin-former, is of value in the treatment of methemoglobinemia.

DR. FISHMAN: Although methylene blue is a methemoglobin-former *in vitro*, it is apparently not as good a methemoglobin-former *in vivo*. The net effect of methylene blue is always a balance between the rate of reduction of methemoglobin to hemoglobin and the rate of oxidation of hemoglobin to methemoglobin; therapeutic doses of methylene blue in patients with large amounts of methemoglobin in circulating blood favor the reduction reaction [*17*].

SUMMARY

DR. RICHARD J. CROSS: Most cases of polycythemia are secondary to a systemic hypoxemia. Perhaps the most clear-cut example of this is found in those who reside at high altitudes. Extensive studies of these subjects have provided considerable information about how they compensate for their environment. The increase in hemoglobin mass provides higher oxygen tensions in the tissues, although it tends to diminish oxygen uptake in the lungs. Recent studies have shown that in residents at high altitudes there is a decrease in the affinity of hemoglobin for oxygen, which serves to raise still higher the oxygen tension in the tissues.

Mild degrees of oxyhemoglobin unsaturation are often encountered in polycythemia vera; their cause is uncertain. Marked unsaturation is more rarely encountered and may indicate that the polycythemia is secondary to damage to the respiratory center or other interference with the normal ventilatory drive. Other causes of polycythemia in the absence of heart or lung disease include methemoglobin, which when present in large amounts can reduce the oxygen supply to the tissues and stimulate erythropoiesis.

Polycythemia vera has long been classified as a myeloproliferative disorder, but great areas of ignorance remain concerning its pathogenesis and etiology. Recent research in this disease has uncovered certain facts of considerable interest. (1) The metabolic activity of the leukocytes of polycythemia vera with a leukemoid reaction differ from the morphologically indistinguishable leukocytes of chronic myeloid leukemia. (2) Polycythemia vera results from an overproduction of erythrocytes of normal life span. (3) The accelerated disappearance of iron from the plasma of patients with polycythemia vera is probably related to erythroid activity, increased iron demand and possibly to iron deficiency; whether other factors are involved is not known. (4) The observation of chromatographic differences in the whole blood of patients with polycythemia may provide some interesting leads in the study of the pathogenesis of this disorder.

Considerable evidence has recently been uncovered which suggests that the formation of red blood cells may be under hormonal control, but no clear-cut proof of this hypothesis has yet been adduced. Current research in this area seems to indicate that erythropoiesis is an enormously complex process of which we have little understanding at present.

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Case Reports

Chronic Idiopathic Immunoneutropenia*

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THE purpose of this report is to describe a patient who has chronic idiopathic neutropenia and to present certain studies which indicate that this is caused by an immune mechanism. Neutropenia has been reported to be caused by leukocyte agglutinins in a variety of circumstances [*1–3*]. Many of these clinical situations appear to be the counterpart of diseases of the red cells and platelets which have an immunologic basis. Thus Moeschlin and coworkers have described a patient with pancytopenia secondary to primary atypical pneumonia in whose serum cold agglutinins were demonstrated for leukocytes as well as for red cells. The same author has presented data [*5*] to indicate that the neutropenia caused by pyramidon is due to an immune mechanism, not unlike that by which fuadin® produces an anemia and sedormid® and quinidine [*8*] cause thrombocytopenia [*8*]. Other authors [*9,10*] have reported an idiopathic neutropenia which parallels idiopathic hemolytic anemia and thrombocytopenia purpura. Various combinations of these clinical entities have been reported.

CASE HISTORY (FIG. 1)

This twenty-one year old white woman was first admitted to St. Mary's Hospital on August 6, 1951 because of a urinary tract infection. Past history revealed that she had had the usual childhood diseases and in addition had had a bout of pneumonia eight years prior to this admission. During the year preceding admission she had had recurrent episodes of stomatitis characterized by crops of aphthous ulcers. These occurred about every one or two months. The physical examination was within normal limits except for an initial temperature of 101°F. and some tenderness in the lower part of the abdomen. There were no enlarged lymph nodes; the spleen and liver were not palpable. The laboratory work revealed a white blood cell count of 1,200 per cu. mm.; the differential count showed polymorphonuclear leukocytes, 5 per cent; metamyelocytes, 15 per cent; lymphocytes, 61 per cent; monocytes, 16 per cent; and basophils, 3 per cent. The red cell count was 4,200,000 per cu. mm.; hemoglobin was 14.0 gm. per cent and the hematocrit was 40; the platelet count (indirect method of Dameshek) [*11*] was 420,000 per cu. mm.; the gamma globulin [*12*] was 0.75 gm. per cent. The blood type was ORh+. The red cell count, hemoglobin, hematocrit and platelet count were repeated on numerous occasions during the period in which the patient was under our observation and they were always within normal limits. The urinalysis showed a specific gravity of 1.015, the reaction to litmus paper was alkaline; there was 3 plus albumin and the microscopic study of the sediment after centrifugation revealed many white and red blood cells. On culture of the urine Escherichia coli was grown; inoculation of three guinea pigs with urine failed to induce tuberculosis in any of the animals. Aspiration of material from the bone marrow revealed normal cellularity and cytology of the elements except for the fact that the white cell series showed no maturation beyond the stage of metamyelocytes. Cystoscopic examination of the bladder disclosed evidence of acute cystitis; retrograde pyelography showed normal configuration of the renal calices, pelves and ureters on either side. The symptoms of cystitis responded promptly to antibiotic therapy.

The second admission to the hospital was on June 20, 1952, again for a urinary tract infection. Her health during the interval had been good except for several episodes of stomatitis. The physical examination was again within normal limits except for tenderness over the lower part of the abdomen. The urinalysis revealed the presence of albumin and the sediment contained red and white blood cells; culture again yielded E. coli. The white blood cell count was 2,300 per cu. mm., the differential count showed polymorphonuclear leukocytes, 20 per cent; metamyelocytes, 14 per cent; lymphocytes, 62 per cent; monocytes, 2 per cent; eosinophils, 1 per cent; basophils, 1 per cent. The remainder of the laboratory work was within normal limits. The symptoms cleared promptly with antibiotic medication.

Following discharge from the hospital the patient

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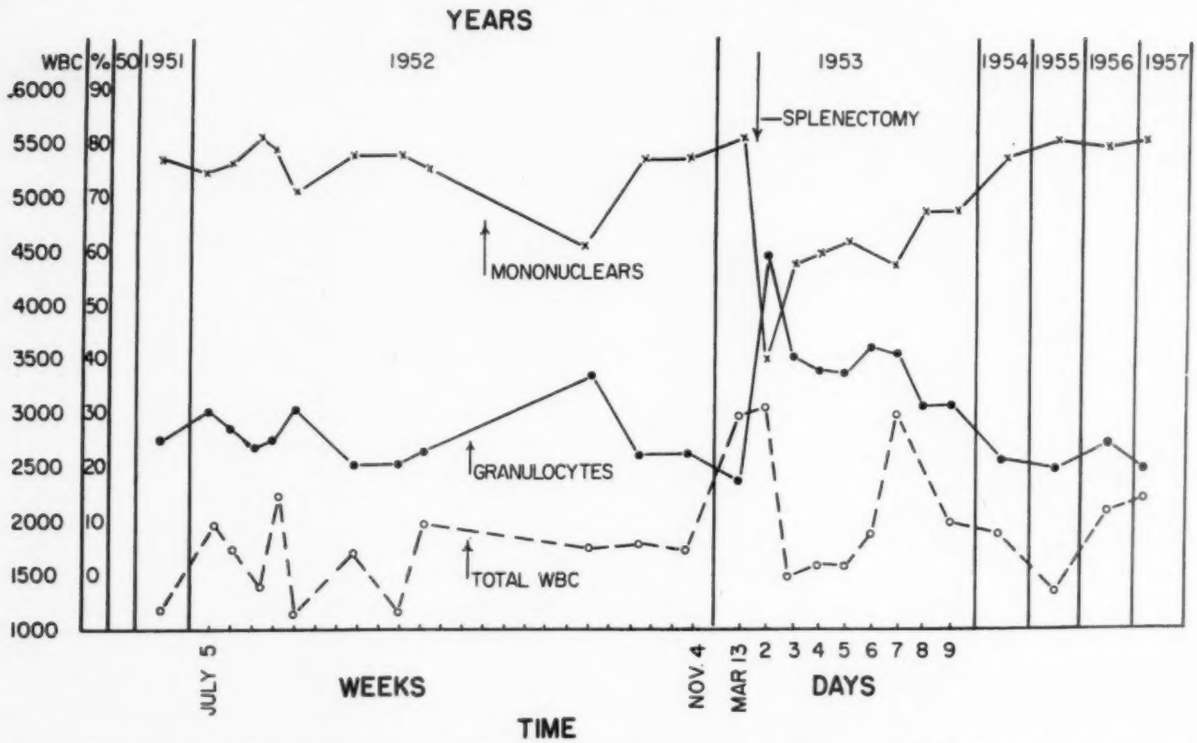

FIG. 1. The total white blood cell count and percentage of neutrophils and mononuclear cells are charted for the period of observation. The blank column for 1950 denotes that the patient's symptoms would indicate that the disease was present during that year although the patient was not under observation.

again felt well except for recurrent attacks of stomatitis. The following courses of medication were administered on separate occasions without effect on the neutropenia: folic acid, 15 mg. daily, for two weeks; vitamin B_{12} 100 μg. per week, for ten weeks; cortisone, 300 mg. for one day, 200 mg. the second day and 100 mg. daily, for a total of ten days.

The patient was again admitted to the hospital on April 13, 1953 for removal of the spleen. She had no symptoms at this time and, as previously noted, the physical examination was within normal limits. The spleen was not palpated. On admission the white blood cell count was 2,100 per cu. mm.; the differential count showed granulocytes, 30 per cent; bands, 10 per cent; lymphocytes, 54 per cent; monocytes, 2 per cent; and eosinophils, 4 per cent. The remainder of the laboratory results were within normal limits.

Splenectomy was performed on April 14, 1953 and the patient received 1,000 cc. of blood during the operation. The spleen was normal on gross and microscopic examination. The total white count remained about the same following surgery although there was an increase in granulocytes for a few days. (Fig. 1.) Thereafter the differential count returned to its previous levels.

The course of her illness since the splenectomy has not been altered. She has recurrent attacks of stomatitis and occasional bouts of pharyngitis, but she has had no further urinary infections. She was married in the fall of 1953 and has lived a normal life; there has been no pregnancies to date.

METHODS

All tests for leukocyte agglutination and the transfusion experiment were performed with blood drawn from the patient prior to the transfusion which she received during the operation. All white blood cell counts were performed using two white cell pipettes with balanced correction factors; two chamber counts were made on fluid from each pipette.

Plasma was used in testing for leukocyte agglutinins. This was done with sterile technic and was stored at —20°c. until used. Sequestrene was used as an anticoagulant throughout. All glassware was siliconized and the metal parts were coated with arquad each time the equipment was used. The silicone was stripped from the glassware after each usage by soaking in 10 per cent sodium hydroxide solution. Blood was collected for the preparation of white cells in the following manner: A needle was inserted in the antecubital vein and blood was allowed to flow up to the mark of a 15 ml. centrifuge tube containing 1 ml. of 1 per cent sequestrene and 1.25 ml. of dextran with an intrinsic viscosity greater than 0.41. The centrifuge tube was covered with parafilm and in-

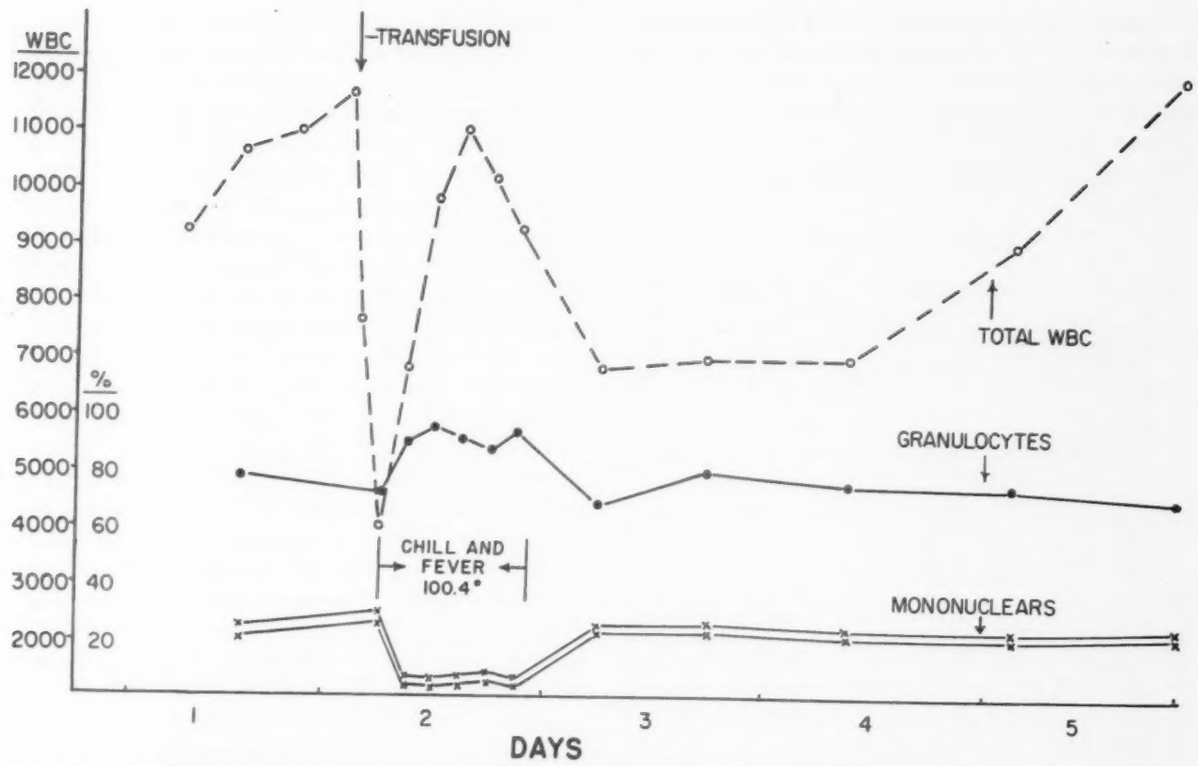

FIG. 2. The total white blood cell count and percentage neutrophils and mononuclear cells of a normal recipient are charted before and after the infusion of 300 ml. of the patient's plasma.

verted gently three times. The blood was allowed to settle for about thirty minutes and the supernatant was removed. The white cell counts were usually between 10,000 and 15,000 per cu. mm. The test was performed by mixing 0.10 ml. of white cell suspension with 0.10 ml. of test plasma in a 10 by 75 mm. serologic test tube. This was incubated in a water bath at 37°C. and read after one hour. The leukocytes were resuspended after incubation by shaking the test tube quickly about six times. A drop was poured on a glass slide and allowed to flow out in a thin layer by tilting the slide. The slide was studied for white cell clumping under the low power of the microscope either with reduced light or with phase microscopy.

Six hundred ml. of the patient's blood was withdrawn into acid-citrate-dextrose solution (U.S.P.) in a donor blood flask. The plasma was separated by centrifugation and was transferred to a donor bottle using sterile precautions. This plasma (about 300 ml.) was transfused into a recipient of the same major blood type (O+). A Coombs' test performed on the red cells of the recipient after incubation with the patient's plasma was negative.

The plasma of twenty-four normal young non-immunized (i.e. nulliparous, non-transfused) donors was tested against white cell suspensions of seven normal donors. These tests were performed on two and sometimes three occasions and were considered positive only when agglutination was present on two or more determinations.

RESULTS

The patient's plasma was tested against the compatible (type O) white cell suspensions of twelve donors; each test was performed on a different day. Ten of twelve tests were positive for leukocyte agglutinins.

The effect of a transfusion of the patient's plasma on the total and differential white blood cell counts of a normal recipient is charted in Figure 2.

The results of the study of leukocyte agglutinins in normal individuals of a compatible blood group are summarized in Table 1.

COMMENTS

The leukocytes are the last of the formed elements to be studied immunologically. The reason for this is quite apparently the difficulty in preparing adequate white cell suspensions in that injury to the leukocyte results in stickiness of the cell and a false positive agglutination reaction. Various methods have been used to prepare

a white cell suspension which is relatively free of red cells and platelets without causing injury to the white cell. Some investigators have prepared the suspension from the buffy coat after centrifugation [*5*]. Others have used flotation technics [*13*] or methods to increase sedimentation of the red cells by rouleaux formation [*14*]. Moeschlin and Schmid used heparin as an anticoagulant and Dausset, Nenna and Brecy [*1*] used defibrinated blood. In our hands centrifugation, heparin, and attempts to defibrinate with glass beads all caused injury to the white cell as evidenced by a non-specific stickiness. Sequestrene appeared to be the best anticoagulant for preventing non-specific clumping; dextran was used as a sedimenting agent for red cells. The suspensions of white cells produced by this method do not clump spontaneously. The leukocytes are irregular in outline, undergo active amoeboid motion, are capable of erythrophagocytosis, and in general appear to be viable. There are, however, a certain number of disadvantages to this method. The fact that sequestrene binds the divalent cations and thus inhibits complement must be considered a major defect. In addition, this technic does not permit concentration of the white cells and thus makes it difficult or impossible to test the leukocytes of patients who have a marked leukopenia. Another disadvantage lies in the fact that no provision is made for washing the white cells; one is thereby unable to do a Coombs' antiglobulin test.

CELL DONORS

PLASMA DONORS		I	II	III	IV	V	VI	VII
	TYPE	O+	O+	A+	A+	A+	B+	B+
1	O+	0	0					
2	O+	0	0					
3	O−	0	0					
4	O+	0	0					
5	O+	0	0					
6	O+	0	0					
7	O+	0	0					
8	O+	0	0					
9	O+							
10	O+							
11	A+	0	0	0	0	0		
12	A+	0	0	0	0	0		
13	A+	0	0	0	0	0		
14	A+	+	0	0	0	0		
15	A+	0	0	0	0	0		
16	A+	0	0	0	0	0		
17	A+		0	0	0	0		
18	A+	+	0	0	0	0		
19	A+	0	0	0	0	0		
20	B+	0	0				0	0
21	B+	0	0				0	0
22	B+	0	0				0	0
23	AB+	0	0	0	0	0	+	0
24	AB+	0	0	+	0	0	+	0

TABLE 1. This table shows the results of the tests for leukocyte agglutinins in plasma from normal individuals using white cell suspensions from normal donors. The plasma and white cell suspensions tested are compatible for the A, B and O blood groups. Agglutination is indicated by +, O denotes no agglutination; a blank space means the test was not performed.

The presence of leukocyte agglutinins in the normal population has been the subject of a number of investigations. It would, of course, be important to differentiate the agglutinins found in a patient with neutropenia from naturally occurring leukocyte agglutinins. Goudsmit and Van Loghem [*2*] tested the serums of 100 healthy donors against type O white cells and found weakly positive clumping reactions in ten instances. Dausset, Nenna and Brecy [*1*] on the other hand studied the serums of 300 normal donors for the presence of agglutinins to compatible leukocyte suspensions and failed to demonstrate any. Butler [*15*] reported six instances of leukocyte agglutinins in eighty-four tests in which the cells and plasma were compatible for the ABO blood groups. A number of studies have been performed in which normal white cell suspensions were tested against normal plasma without regard for compatibility with respect to the A, B and O blood groups. Dausset [*17*] cited some unpublished experiments in collaboration with Van Loghem in which combinations of fifty normal serums and fifty corresponding varieties of white cells were tested for leukocyte agglutination. They observed frequent but inconstant agglutinations in the combinations which were incompatible for the ABO blood groups. Moeschlin and Schmid [*16*] tested blood samples of thirty persons in 380 leukocyte serum combinations with regard to the A, B and O blood groups. They noted leukocyte agglutination in sixty cases of the 380 combinations. Butler [*15*] tested seventy-six combinations of white cells and plasma and on repeated tests noted that twenty-four of the combinations were positive. He observed that these clumps were composed of red and white cells as opposed to the pure white cell clump formed in the combinations which

were compatible for the A,B,O blood groups. Further study revealed that these clumps were the result of erythrophagocytosis with subsequent clumping of the erythrophagocytes. Swisher [*18*] has reported another type of mixed red and white cell clump as a result of incubating incompatible serums and white cell suspensions. This seems to be due to a non-specific adherence between the red and white cells. It appears, then, that a small percentage of normal persons may have leukocyte agglutinins for certain compatible white cells. Incompatible white cell suspensions cause a mixed red and white cell clump which is not indicative of leukocyte agglutinins.

The results of the *in vitro* tests in this case leave little doubt that we are dealing with an abnormal leukocyte agglutinin. The white cells from twelve compatible donors resulted in ten positive agglutination reactions. The control studies revealed only five positive reactions in eighty-seven tests, and in only one instance did a plasma contain agglutinins for more than one donor (plasma 24, Table 1).

We have been able to collect from the literature 174 cases in which leukocyte agglutinins have been demonstrated [*2,3,9,10,19–27*]. The relationship between the leukocyte agglutinins and the leukopenia in these cases, however, is far from clear. While it is true that the majority of cases are leukopenic (this was specifically mentioned in 138 cases), these are for the most part cases of chronic anemia which have required multiple transfusions. It is difficult to be certain about the number of transfusions in all cases because the authors are not always explicit on this point. Dausset [*19*] has estimated, however, that only about ten cases of neutropenia with leukocyte agglutinins have been reported in individuals who had not had transfusions. In addition most authors have been unable to demonstrate agglutinins against the patient's own white blood cells (autoagglutinins). It is thus not conclusively proved that the leukocyte agglutinins in these cases are responsible for the leukopenia but may only represent isoimmunization by the white cells received in blood transfusions. On the other hand, Andre, Dreyfuss and Bessis [*21*] have demonstrated agglutination of the patient's own white cells by the pathologic serum in one instance. Bessis [*37*] has also observed that the leukocytes of patients with immunoneutropenia are ingested by normal leukocytes *in vitro* and he believes that this opsonin-like effect of the patient's serum is evidence of an autoagglutinin. In Martensson's [*9*] case the leukopenia became more severe for five days following his patient's first transfusion of blood. This author suggests that a co-factor may have been contributed in the transfusion which enhanced the action of the leukocyte agglutinin. Perhaps the absence of this co-factor in some way interferes with the demonstration of autoagglutination. On the other hand, the lack of this demonstration may be due to technical difficulties. It is possible that the lack of neutrophils in the white cell suspension caused by the marked leukopenia makes it difficult to demonstrate agglutination. In spite of this difficulty, however, if agglutinins do cause this disease they must somehow affect the patient's own white cells. Presumably, the same theory of autoimmunization which is used to explain the pathogenesis of hemolytic anemia applies to this disease. Although there is considerable controversy about this, the present thought seems to be that some injury (virus, drugs) to the red cell uncovers an antigen which is new to the organism. This acts as a stimulus for the production of antibodies against the red cell on which this antigen resides. Recent investigations have identified the relationship of some of these antibodies to the specific blood group antigens [*38*].

Several authors [*4,5,25,28–30*] have attempted to demonstrate leukocyte agglutinins by transfusing blood from the patient into a normal donor. In all instances reported (except one in which it is not specified [*25*]) this has resulted in a transient fall in white blood cell count. The transient nature of the fall in white blood cell count is so similar to the so-called pyrogen reaction described by Bennett and Beeson [*31*] that some have questioned the validity of this observation. In addition, in the present report and in one other [*45*] a chill and fever were noted in association with the transfusions. On the other hand, it seems unlikely that a number of similar experiments carried out in different hospitals should be caused by an exogenous pyrogen. Furthermore, it is possible that the transient nature of the fall in white count is not characteristic of pyrogen reactions alone. The experiments of Craddock and Lawrence [*32*] have demonstrated that there is a very large reserve of white cells which can be quickly mobilized. A correspondingly large insult would thereby be required to induce a prolonged fall in

white blood cell count. One might expect that the inoculation of a small dose of a leukocyte agglutinin could produce a transient fall in the white count by a mechanism different from the pyrogen reaction. Some support for this reasoning might be found in the rise in recipient's granulocytes immediately following the drop in total white cell count. (Fig. 2.) Another possible explanation combines the ideas that the transient fall in white cells is on an immune basis as well as due to a pyrogen reaction. Atkins and Wood [*33*] have pointed out that the regular occurrence of leukopenia following the injection of a bacterial pyrogen suggests that injury to leukocytes may release tissue pyrogen. It is possible that in the present instances the leukocyte agglutinin by injuring the leukocytes releases tissue pyrogen which causes the fever and contributes to the leukopenia.

Another possible method of determining whether or not this disease is caused by an immune mechanism might be to determine the white blood cell count of the offspring of this patient during the first few hours of life. The finding of leukpenia at this time might lend considerable weight to the theory that the cause is leukocyte agglutinins. Similar findings of thrombocytopenia in the newborn of mothers with idiopathic thrombocytopenia have been helpful in establishing a similar etiology in that disease [*35*]. The antiglobulin consumption test [*36*] and tests for leukoprecipitins [*37*] have been used by other authors in studies of this type of disease but we have had no experience with these methods.

Moeschlin [*39*] has studied the mode of destruction of white cells by injecting rabbits with antirabbit leukocyte serums. He demonstrated masses of agglutinated leukocytes plugging the capillaries of the lungs in animals who were sacrificed during the leukopenic phase following injection of the antiserum. The conclusion was that the leukopenia is caused by agglutination of the circulating leukocytes with subsequent destruction and removal from the peripheral blood in the capillaries of the lung.

In the differential diagnosis of this entity one must consider the various diseases which are characterized by a chronic leukopenia, including other diseases in which leukocyte agglutinins have been demonstrated. The latter include virus pneumonia, infectious mononucleosis, Felty's syndrome, lupus erythematosus, splenic tuberculosis, plasmacytoma, paroxymal nocturnal hemoglobinuria, acquired hemolytic anemia, aleukemic leukemia, paroxysmal cold hemoglobinuria, as well as drug induced leukopenia on an immune basis and multiple transfusions [*2,40,41*]. Cases of chronic leukopenia do not always fall into clearly defined categories but the following are some which have been recognized: periodic diseases [*42*], primary splenic neutropenia [*43*], chronic hypoplastic neutropenia [*44*], chronic granulocytopenia in childhood [*45*], familial neutropenia [*46*] and agammaglobulinemia [*47*].

Many of the diseases mentioned in the differential diagnosis need not be dealt with in any but a cursory fashion. Others must be considered in more detail. On repeated questioning the patient denied the ingestion of drugs of any kind. The long contact with the patient and her family, including visits to the home environment, make it seem reasonably certain that this cause can be excluded. The length of follow-up would tend to rule out certain illnesses such as leukemia, lymphoma, plasmacytoma, chronic granulocytopenia of childhood, infectious mononucleosis, and this factor in addition to repeatedly negative L. E. cell preparations seems to exclude lupus erythematosis. There has been no evidence of arthritis to indict Felty's syndrome. The failure of splenectomy to alleviate the condition would exclude hypersplenism and microscopic examination of the spleen did not substantiate a diagnosis of tuberculosis. The constancy of the leukopenia ruled out periodic disease. The bone marrow studies, lack of familial distribution and presence of gammaglobulin in the patient's plasma militated against the diagnoses of chronic hypoplastic leukopenia, familial leukopenia and agammaglobulinemia, respectively.

The patient's symptoms date back to 1950 and she has been under observation since 1951. The course has been that of a benign disease characterized only by repeated infections which have been easily controlled for the most part by antibiotics. The patient has lived a normal life in spite of her illness. Categorizing a case as idiopathic is always somewhat less than satisfactory in that it is done by exclusion. Yet cases of chronic neutropenia of this type are unusual. The follow-up observations of other cases of idiopathic immunoneutropenia have not been sufficiently long to establish any characteristic clinical features which might be associated with this entity. On clinical grounds it resembles the

five cases of chronic idiopathic neutropenia reported by Adams and Witts [*48*]. The infections in the present case seem milder but that may well be due to the greater availability of antibiotics. As in the cases of these authors, splenectomy was of no avail but the prognosis appears to be good.

SUMMARY

A case of leukopenia which has been followed for five years is reported. *In vitro* and *in vivo* tests indicated the presence of leukocyte agglutinins. The immunologic basis is not conclusively proved but there seems to be a parallel between this disease of white cells and idiopathic hemolytic anemia and thrombocytopenic purpura.

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Acute Renal Failure Responding to Potassium Replacement Therapy*

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In recent years there has been a considerable growth of information concerning the role of body potassium in normal and diseased states. The case to be presented is believed to represent a generally unappreciated aspect of potassium derangement, namely the association of hypokalemia with acute renal insufficiency.

CASE REPORT

A. R., a fifty-two year old odd-jobber, was admitted to the Strong Memorial Hospital on March 2, 1953, in an intoxicated state. Except for chronic leg ulcers, his health prior to admission was unremarkable until a bout of gradual ankle swelling prompted hospitalization five months before. At that time, pitting leg edema associated with chronic leg ulcers was noted. The blood pressure was 164/96 mm. Hg. The urine was grossly bloody, acid, and contained albumin, innumerable red and white blood cells and a few granular casts. The urea clearance was 32 per cent of normal and blood serum values including urea nitrogen, sodium, potassium, chloride and CO_2 combining power were normal. The patient was hospitalized for one month, his treatment consisting of a low salt diet, blood transfusion and thiomerin® injections. Following discharge he was given five additional thiomerin injections before he discontinued his visits to the clinic.

Three weeks prior to the present admission the patient stopped eating, sustaining himself entirely by the daily consumption of 15 ounces of wine. He grew weak, and vomiting and diarrhea developed. In the week prior to admission he was passing 3 to 5 non-bloody diarrheal stools a day and having a similar number of vomiting bouts. The precise amount of urine voided is uncertain, but shortly before admission it was again noted to be red in color.

On examination, the blood pressure was 162/80–90 mm. Hg. He was an obese, obtunded individual. The skin was dry and lusterless. The eyes were crusted with a green-colored exudate. The funduscopic examination was obscured but previously known to be within normal limits. The eyeballs were soft and slightly sunken. There was slight pitting ankle edema and minimal hepatomegaly. The upper extremities were tremulous. The urine was bright red and contained albumin and countless red and white blood cells. The stool was diarrheal and continued so throughout the period to be described, there being 2 to 4 daily movements. The hematocrit, hemoglobin and white blood counts were normal. The initial serum chemical determinations are listed in Table 1.

During the first forty-eight hours of hospitalization it was noted that the patient was oliguric despite infusion of 3,100 cc. of fluid. Careful reappraisal of the events prior to admission with respect to injury, trauma or ingestion of possible toxic agents provided no explanation for oliguria. In view of the severe hyponatremia and hypochloremia, a trial infusion of 500 cc. of 2.5 per cent saline solution was given. This produced no perceptible increase in urine flow.

On the third hospital day the pH value of arterial blood measured 7.52. At this time he was given a daily intake of 600 cc. of butter soup mixture, calculated to provide 2,200 calories and containing negligible amounts of potassium. Water intake was restricted to 900 cc. and a daily allowance of 250 mEq. of sodium was provided.

During the second week a repeat infusion of hypertonic saline solution again failed to induce urine flow. The daily urine output remained 100 cc. or less; its specific gravity ranged from 1.015 to 1.019; it was alkaline and free of cellular casts. The supplementary sodium intake was discontinued. Two separate plasma volume determinations using the blue dye method gave values of 3,560 to 3,850 cc.

Despite evidence of progressive uremia, the sodium, chloride and CO_2 values showed a slow restitution toward normal. The serum potassium levels remained below normal, however, and the electrocardiogram showed changes consistent with hypokalemia. (Fig. 1.) A muscle biopsy obtained on the seventh hospital day demonstrated the low normal potassium concentration of 45 mEq./100 gm. of fat-free solids. On the twelfth hospital day a trial oral dose of 20 mEq. of potassium was given. Because of persistent vomiting, it was uncertain how much of this was retained. The serum potassium level and electrocardiogram remained unchanged. The following day a total of 75

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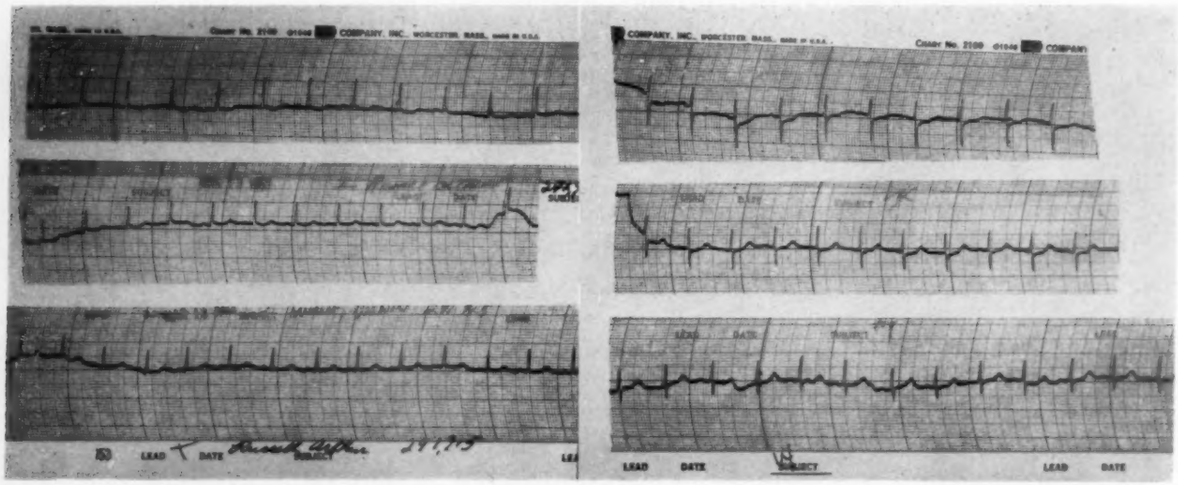

FIG. 1. Electrocardiogram. Lead 1 on the left and V4 on the right. From above downward the eighth, fourteenth and seventeenth hospital day. The upper tracing demonstrates changes characteristic of hypopotassemia consisting of sagging, depressed S-T segments and low-amplitude, flattened T waves. Note the complete resolution of these abnormalities in the two lower tracings which were taken subsequent to potassium therapy.

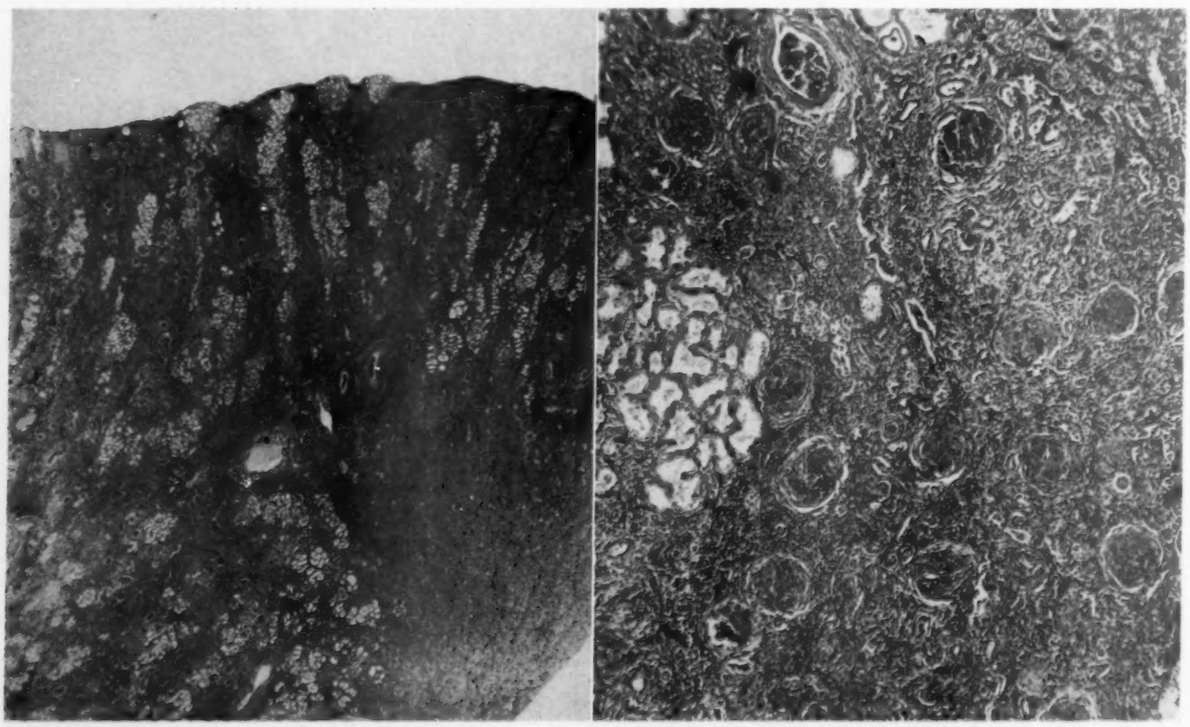

FIG. 2. Microscopic picture of the kidney (hematoxylin and eosin stain). Low power view (left) and high power view, original magnification × 625 (right). Note islands of dilated tubules, partially and completely obliterated glomeruli with adhesion of capillary tufts, thickening of capsule, and crescent formation as well as arteriolar thickening.

mEq. of potassium was carefully administered in two separate intravenous infusions. There were no adverse effects nor any immediate changes in the serum potassium or electrocardiogram. By the next day, however, the serum potassium level had risen to the normal range where it remained for the subsequent hospital period. The distinct improvement in the electrocardiogram is noted in Figure 1. The urinary output of sodium and potassium during the fourteenth to seventeenth hospital days is noted in Table 1. On the fifteenth hospital day the urine volume exceeded 300 cc. for the first time in two weeks and subsequently remained at normal levels.

With restitution of normal urine flow, the uremia gradually lessened and the serum electrolytes assumed a normal pattern. The cellular elements gradually

TABLE I

LABORATORY STUDIES DURING HOSPITALIZATION

Hospital Day	Fluid		Serum (mEq./L.)				Blood Urea Nitrogen (mg. %)	Urine	
	Intake (cc./24 hr.)	Output (cc./24 hr.)	Sodium	Potassium	Chloride	CO_2		Sodium (mEq./24 hr)	Potassium (mEq./24 hr.)
1	....	....	111	3.65	43	41	74	..	..
2*	2000	100	105	4.5	48	44	...	..	..
3	1100	400	113	3.5	54	35	74	..	..
4	900	250	118	3.1	58	..	78	..	..
5	900	50	118	3.5	61	32	79	..	..
6†	950	50	119	3.7	68	35	88	..	..
7	900	....	121	3.1	68	36	90	..	..
8	875	60	128	3.4	77	28	100	..	..
9	790	100	124	3.6	75	27	100	..	..
10	775	95	127	3.2	76	28	98	..	..
11	900	100	126	3.4	75	26	110	..	..
12‡	575	140	130	3.0	75	28	108	..	..
13§	1000	215	132	3.3	77	29	113	..	..
14	0	245	133	4.3	83	27	124	12	9
15	725	400	133	5.0	84	26	126	23	16
16	760	350	136	4.9	83	28	132	12	14
17	1750	600	137	4.5	...	..	...	18	24
18	2250	650	140	6.2	85	29	128	..	..
19	3000	600	133	5.7	86	30	125	..	..
20	365	1400	134	5.4	...	31	111	..	..
21	800	500	133	5.2	82	28	114	..	..
26	....	700	138	5.2	93	29	89	..	..
29	....	750	143	4.4	95	32	69	..	..
40	....	....	135	5.3	102	26	52	..	..
52	....	....	136	5.3	101	26	69	..	..
71	....	....	139	5.1	91	28	73	..	..

NOTE: The figures for fluid intake include both oral and intravenous intake.

* = Intravenous infusion of 500 cc. 2½ per cent saline.

† = Intravenous infusion of 200 cc. 5 per cent saline.

‡ = Oral administration of 20 mEq. of potassium chloride.

§ = Intravenous infusion of 75 mEq. of potassium chloride.

decreased in the urinary sediment. There was no definite period of diuresis during recovery.

The patient remained hospitalized for two months and his course was complicated by the occurrence of a urinary tract infection, pericarditis, heart failure and anemia. He gradually improved with the aid of digitalization, low salt diet, transfusion and orally administered iron. When discharged on May 4, 1955, he was still moderately anemic and mildly azotemic, and the blood urea nitrogen ranged between 40 to 70 mg. per cent.

The patient's status remained delicate during the next three months, in which time he was followed in the outpatient clinic. He grew discouraged and was lost to follow-up until one year later when readmitted in a comatose and obviously terminal state. He died within twenty-four hours of this final admission.

At autopsy, performed on October 28, 1954, the immediate cause of death was noted to be a massive cerebral hemorrhage. The general findings consisted of encephalomalacia, pneumonia, chronic passive pulmonary congestion and emphysema. The left kidney weighed 100 gm. and the right 90 gm. Both kidney surfaces were diffusely scarred. The cortex was 4 mm. in width and streaked with white strands. The microscopic picture (Fig. 2) demonstrated chronic glomerulonephritis as well as arterial and arteriolar nephrosclerosis. There were no obvious findings related to the prior episode of acute renal failure.

COMMENTS

The present study afforded an unusual opportunity to observe a patient in whom acute renal failure appeared to be related to potassium depletion. The defense of this position rests on the fact that after fourteen days of oliguria, unresponsive to initial hydration or treatment

with hypertonic saline solution, normal urine flow was restored on a restricted fluid intake within forty-eight hours of the administration of 95 mEq. of potassium. The restoration of urine flow was associated with elevation of the serum potassium level to normal and resolution of characteristic electrocardiographic abnormalities. Neglecting losses other than urinary excretion, these changes represent a corrected deficit of 70 mEq. of body potassium. This relatively small deficit was not reflected in the muscle biopsy. At autopsy, eighteen months later, the characteristic changes of chronic glomerulonephritis were noted, but there were no findings that could be clearly related to the prior episode of acute renal failure.

Potassium deficiency does not ordinarily occur as an isolated event. Even when induced under more ideally controlled experimental conditions [*1,2*], it tends to be associated with a hypochloremic alkalosis such as was present in the subject of this report. It has recently been noted that oliguria may be a part of the state induced by experimental potassium depletion in previously healthy rats [*1*]. Also, it appears that potassium depletion in human subjects may produce some type of primary renal damage although this damage has not been associated with oliguria [*3*]. The absence of acute renal failure in the many reported instances of potassium depletion in man suggests that this rare concurrence depends on some special circumstance. In this respect it is noteworthy that, in the present report, the patient's recovery was associated with correction of a critically small potassium deficit.

The mechanism of acute renal failure is uncertain even in those cases in which associated structural damage is well documented, such as shock, trauma and transfusion reactions. Oliver [*4*] favors the concept of abnormal back diffusion in the disrupted tubule although this view is admittedly of a speculative nature. The problem is of very special interest when associated with electrolyte depletion, however, because in these instances there is no proof of an acute attendant structural alteration. Schroeder's [*5*] report is the first clear statement that a depletion of electrolyte could be responsible for acute renal failure. He noted that prolonged use of mercurial diuretics along with salt restriction could at times deplete the body chloride and induce oliguria. In all the reported cases there was pre-existent cardiovascular or chronic renal disease. The administration of hypertonic salt solution afforded dramatic relief in a number of instances. Such rapid restitution of urine flow suggests that oliguria was dependent on some functional failure.

McCance [*6*] induced acute sodium depletion in human subjects and found that an administered water load was excreted abnormally. This abnormal response was attributed to a demonstrated impairment of glomerular filtration. However, examples of chronic edematous states with low serum sodium levels but with normal urine output are a common experience. The intimate relationship which is presumed to exist between the tubular transport mechanism for sodium and the handling of water, potassium and hydrogen ion would tend to implicate the tubule as the likely site of functional failure. The present report merely serves to call attention to the finding that potassium depletion may be a factor in certain cases of unexplained acute renal failure.

SUMMARY

A case of acute renal failure associated with potassium depletion and apparently responding to potassium replacement therapy is reported.

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Fulminating Uremic Pneumonitis Associated with Acute Ischemic Nephropathy (Lower Nephron Nephrosis)*

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IN a recent article on the subject of uremic pneumonitis, Hopps and Wissler [*1*] especially emphasized the frequency of this lesion, noting it in 62 per cent of 107 autopsy cases of uremia. They also pointed out its relative frequency in cases of uremia of short duration, the lesion being present in sixteen (84 per cent) of nineteen patients who had uremia for less than one week. The case to be described here appears to be an especially fulminating one, apparently developing within two days of the onset of acute renal shutdown, and resulting in the patient's death approximately three days later.

CASE REPORT

A seventy-five year old white housewife was admitted to the Mississippi Baptist Hospital on the evening of September 16, 1956, complaining of epigastric cramping pain. The distress, which had been present since it awakened her from sleep on the night of September 13, was described as constant, moderate and occasionally "acute," radiating into the left anterior chest as well as to the left subscapular region. There were no associated gastrointestinal complaints, the patient having continued to eat heartily, although feeling rather full postprandially. There had been no chills, fever or jaundice. The symptoms becoming progressively more intense, the patient sought medical aid after three days.

Previous similar attacks were denied. This admission was the first hospitalization in her life. She had been told by a physician in another state that she had a "diseased heart" (no cardiorespiratory symptoms could be elicited by our questioning) and an "acid stomach" because of occasional heartburn, which she relieved symptomatically with baking soda. The only other finding of note in the systemic review was that for fifty-five years an indefinite mass had been present about the umbilicus which occasionally "stuck out like an egg." The past history and family history were non-contributory.

Examination revealed the patient to be an alert, cheerful, cooperative elderly woman, well-developed and well-nourished, who did not appear to be acutely ill. Her blood pressure was recorded as 140/80 mm. Hg. The respiratory rate was 20 per minute; the pulse rate, 76 per minute; and the temperature, 98.6°F. No abnormalities were found on examination of the head, fundi, neck, heart, lungs and breasts. The abdomen was tender to palpation in the epigastrium, right upper quadrant and periumbilical region. Marked percussion tenderness was noted in the right upper quadrant. No rebound phenomenon was elicited. The liver, kidneys and spleen could not be palpated, nor were any masses evident. Peristalsis was moderately hyperactive. An umbilical hernia, egg-sized, was present. No costovertebral angle tenderness was found. Examination of the rectum, pelvis and extremities revealed no abnormalities.

On admission the hemoglobin was 13.3 gm. per cent and the hematocrit, 42 volumes per cent. The white blood cells numbered 9,100 per cu. mm. with 70 per cent segmented neutrophils, 25 per cent lymphocytes and 5 per cent monocytes. A urine specimen obtained by catheterization had a specific gravity of 1.016, a trace of albumin, no reducing substance and 2 to 6 red blood cells per high power field.

The admission diagnosis was cholelithiasis with cholecystitis.

On September 17, blood chemistry studies found the serum amylase to be 48 units; the total bilirubin, 1.1 mg. per cent; blood glucose, 106 mg. per cent; alkaline phosphatase, 2.5 Bodansky units; thymol turbidity, 0.7 units; and blood urea nitrogen, 12.8 mg. per cent. Roentgen studies the same day failed to visualize the gallbladder twelve hours following telepaque® administration. Films of the upper gastrointestinal tract revealed a small hiatus hernia, but were otherwise normal. An x-ray of the chest showed the aorta to be tortuous, the cardiac size and contour to be normal, and the lungs free of evidence of disease. The patient was advised to have her gallbladder re-

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moved at once. She refused surgery because of her husband's absence on business.

By the morning of September 18 there was temperature of 100°F., distention, nausea, vomiting and marked tenderness in the right upper quadrant of the abdomen with rebound phenomenon. The total white cell count at this time was 20,500 per cu. mm. with 84 per cent segmented neutrophils, 3 per cent neutrophilic bands, 5 per cent lymphocytes and 8 per cent monocytes. It was felt that surgery could not await the husband's return. On consultation, the surgeon agreed that immediate intervention was mandatory.

Surgery was undertaken at 10:00 A.M. on September 18. The gallbladder was removed with relative ease; the pathologist reported diagnoses of "acute cholecystitis and cholecystolithiasis." Anesthesia was completely uneventful. The patient's blood pressure was 120/70 mm. Hg on leaving the operating room. No blood was given during surgery. In the recovery room the blood pressure dropped to 80/0 mm. Hg and the patient became slightly cyanotic. Her pulse rate did not exceed 100 beats per minute; it was stated that the pulse always felt much stronger than the blood pressure might indicate. Supportive measures included 500 cc. of whole blood and 50 cc. of a solution of hydrocortisone administered intravenously, oxygen and vasopressors. The response was slow; the patient was not returned to her room for five hours.

By the morning of September 19 she appeared much better clinically. The blood pressure was 94/60 mm. Hg, and the pulse rate was 90 per minute. The lungs were clear. The patient had become oliguric, however, with a total urinary output of only 255 cc. from 7:00 A.M. September 19 to 7:00 A.M. September 20. The daily urine volumes for the remaining hospital days were 192 cc., 770 cc. and 540 cc. Every effort was made to avoid over-hydration and to keep the electrolytic balance as nearly normal as possible. A blood urea nitrogen was 31.3 mg. per cent. A urine specimen obtained from an indwelling bladder catheter had a specific gravity of 1.017, and showed a trace of albumin. Innumerable red cells per high power field were noted. By the morning of September 20 the carbon dioxide combining power was 45.7 volumes per cent; the chlorides, 469 mg. per cent; the potassium, 4.6 mEq./L.; the sodium 122.9, mEq./L.; and the blood urea nitrogen, 45 mg. per cent.

Respiratory difficulty appeared on the afternoon of September 20, with orthopnea, cough of a hacking nature and mild cyanosis, unaffected by the administration of oxygen by nasal catheter at 8 L. per minute. There was no clinical evidence of congestive failure; an electrocardiogram was interpreted as being within normal limits. Nevertheless, the patient was given digitalis, but it did not affect the patient's condition.

A portable chest x-ray film on the morning of September 21 showed mottled shadows of increased density in both lung fields, extending out from the hilar regions. The blood urea nitrogen had risen to 54.5 mg. per cent. The carbon dioxide combining power had declined to 37.2 volumes per cent. The chlorides were 478 mg. per cent, the potassium was 5.3 mEq./L. and the sodium was 126.7 mEq./L.

Despite the oliguria, the respiratory difficulty entirely dominated the picture by September 22. Subcrepitant and musical rales were apparent in both lung fields without signs of consolidation. The expiratory phase was considerably prolonged. Cyanosis was marked. Breathing was of the "grunting" type. The sensorium became clouded. During the evening small amounts of bloody mucus were obtained on suctioning the trachea. During this entire period the blood pressure remained stable around 98/60 mm. Hg, with no cardiac dysfunction becoming apparent.

Breathing was labored in the extreme on the morning of September 23, with much difficulty and effort in expiration. Only a small amount of mucus could be obtained by tracheal aspiration. The patient's condition progressively declined as breathing became more impaired. Death occurred at approximately 9:30 A.M.

The chief autopsy findings of interest were in relation to the heart, lungs and kidneys. The left pleural cavity contained approximately 1 L. of straw-colored fluid with some strands of fibrin contained therein. The lungs appeared quite voluminous, filling the pleural cavities. The combined weight of the lungs was 1,300 gm. The lower portion of the trachea, the mainstem bronchi and the segmental bronchi were filled with inspissated, rather firm grayish tan material. The pleural surfaces, bilaterally, were grayish-blue to pinkish blue but did not appear atelectatic. They varied from subcrepitant to firm on palpation. On transverse section all lobes presented rather uniform grayish red, moderately firm dry surfaces from which only a moderate amount of foamy fluid could be expressed with rather marked pressure.

Fibrinous pericarditis was present, with a definite membrane of fibrin present over most of the heart. When peeled away this left slightly congested surfaces. The heart weighed 400 gm. There was a moderate degree of calcified atherosclerosis with no significant narrowing of any of the lumens, however, except in the left anterior descending branch. At a point approximately 1.5 cm. from its origin it was narrowed, but not occluded. The endocardium and valves were all essentially negative in appearance.

The kidneys weighed 190 gm. each. The capsules stripped with ease leaving rather smooth, somewhat mottled tan renal surfaces. On transverse section there was some bulging of the cut surface with the cortical margins obscured and the cortex appeared quite pale. In several of the pyramids there were noted small punctate brown dots and linear brownish streaks at the corticomedullary junction.

Microscopically, examination of the heart confirmed the fibrinous pericarditis noted grossly. (Fig. 1.)

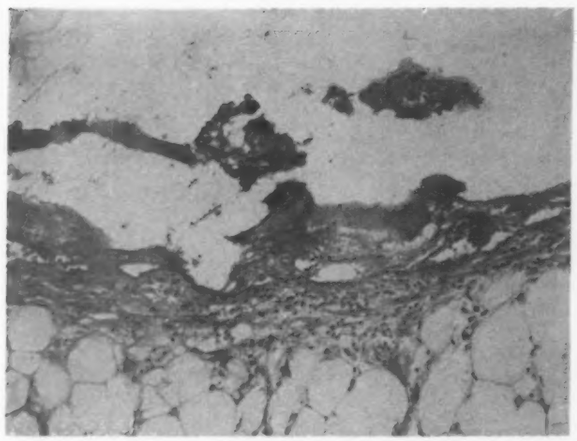

FIG. 1. Epicardial surface showing zone of fibrin deposition with mild inflammatory reaction (uremic pericarditis).

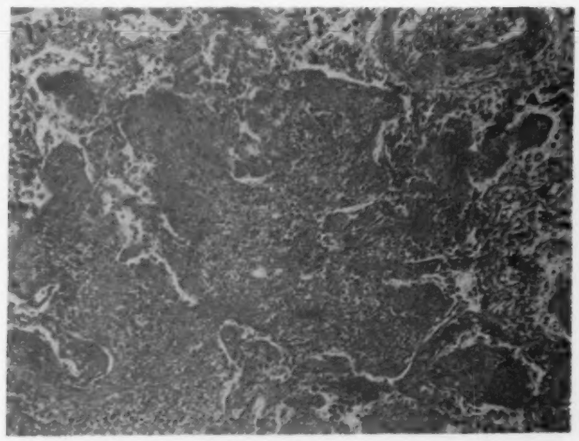

FIG. 2. Section of the lung showing alveoli engorged with massive fibrinous exudate.

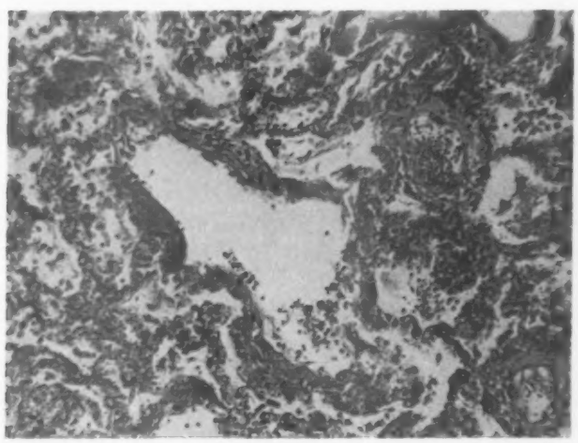

FIG. 3. Section of the lung with hyaline-membrane like appearance of fibrinous pneumonitis.

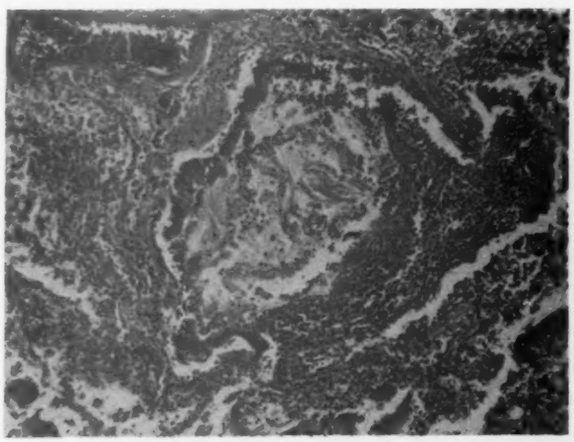

FIG. 4. Small bronchial radicle showing erosion of wall with fibrinous exudate and collection of mucinous material. Evidence of more acute inflammation is noted here.

There was quite a variation in the microscopic picture in various sections of the lungs, but fairly uniformly present were large plugs of fibrinous material within the alveoli. Frequently these were tremendous and distended the alveoli and connected through the pores of Cohn to adjacent alveoli. (Fig. 2.) In most areas there was a relatively scant cellular infiltrate accompanying this process, this being predominantly mononuclear. In other areas there were larger numbers of neutrophils, and in some there was evidence of interstitial pneumonitis in adjacent alveolar septae. Some fields showed fairly widely distended alveoli in which this fibrinous material was applied to the alveolar walls in the form of hyaline-appearing membranes. (Fig. 3.) Sections of small bronchi showed frequent erosion of the bronchial mucosa, a large plug of fibrinous exudate usually being adjacent to the eroded area. (Fig. 4.) Also present in some of the bronchi were fairly large masses of mucinous material with entrapped neutrophils and macrophages. The process in the lung was thought to consist basically of a so-called uremic pneumonitis, the massive fibrin component of the exudate being characteristic of this. A superimposed bacterial infection might also have been present because of the acuteness of the exudate in some areas. This could not be confirmed by bacteriologic studies because of prior arterial embalming.

Sections of the kidneys confirmed the presence of an acute ischemic nephropathy. Many glomeruli were congested. There was considerable interstitial congestion in the pyramidal portions. Pigment casts were present in moderately large numbers, these being reddish brown in color. (Fig. 5.) Frequently associated with these casts were degenerative changes in the tubular epithelium and occasional evidence of erosion of the casts into the interstitial tissue. (Fig. 6.) There were patchy zones of interstitial inflammatory reaction, the cells present being predominantly lympho-

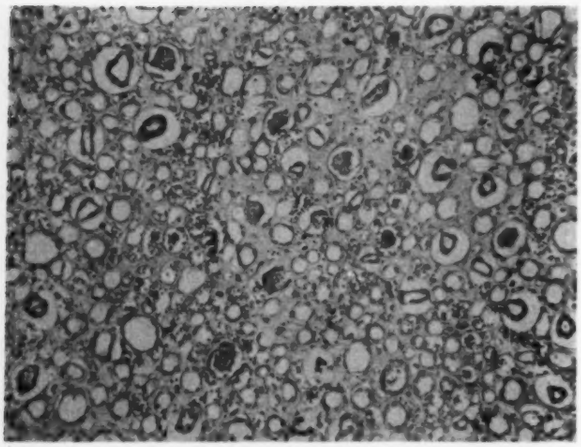

FIG. 5. Numerous pigment casts in collecting tubules of kidney.

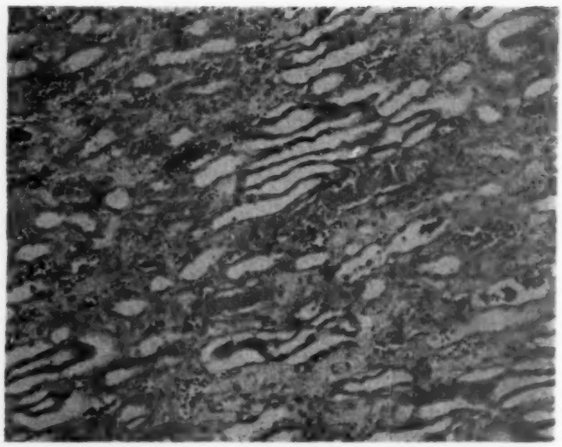

FIG. 6. Large pigment cast with associated tubular necrosis.

cytes and macrophages. No vascular changes suggestive of a pre-existing arteriolar nephrosclerosis were present.

The final pathologic diagnoses in this case were: recent postoperative state following cholecystectomy for acute cholecystitis and cholecystolithiasis; acute ischemic nephropathy (lower nephron nephrosis); uremia (clinical); uremic pericarditis; massive uremic pneumonitis, bilateral; inspissated secretions in bronchial tree; fatty metamorphosis, liver, with occasional zones of focal necrosis; umbilical hernia with incarcerated portion of omentum; diverticulosis, colon; fibrous pleural adhesions, left.

SUMMARY

A case of a seventy-eight year old woman in whom shock and acute ischemic nephropathy developed following cholecystectomy for acute cholecystitis and cholecystolithiasis is presented. The blood urea nitrogen, which was normal prior to surgery, rose to 54.5 mg. per cent by the third postoperative day. During the final two days of this patient's course respiratory difficulty dominated the clinical picture. This was characterized by an asthmatic type of respiration with subcrepitant and musical rales and expiratory difficulty. Autopsy revealed a fibrinous pneumonitis characteristic of those cases previously described as "uremic pneumonitis." Concomitantly, a fibrinous pericarditis compatible with uremic origin had developed.

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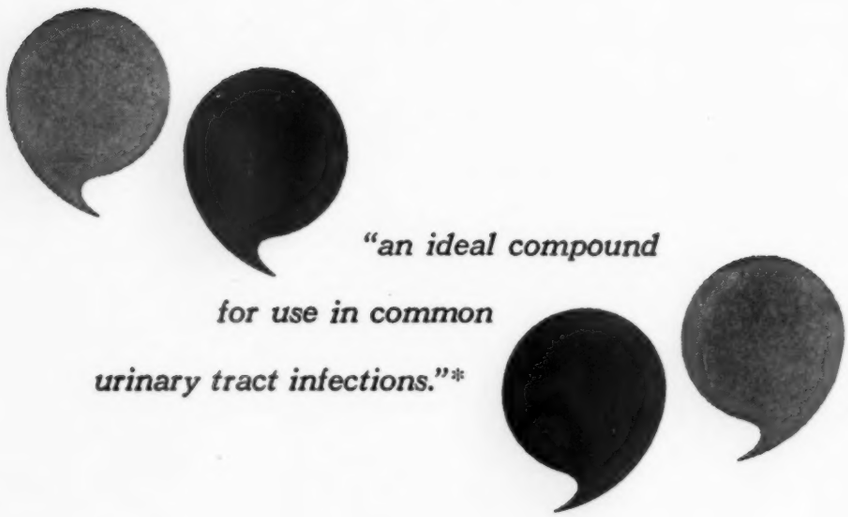

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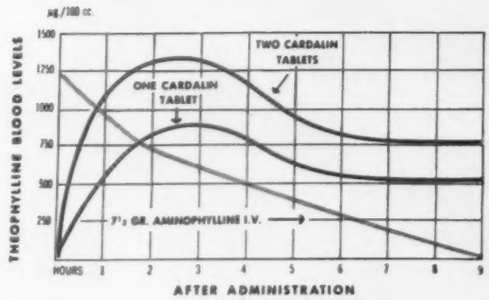

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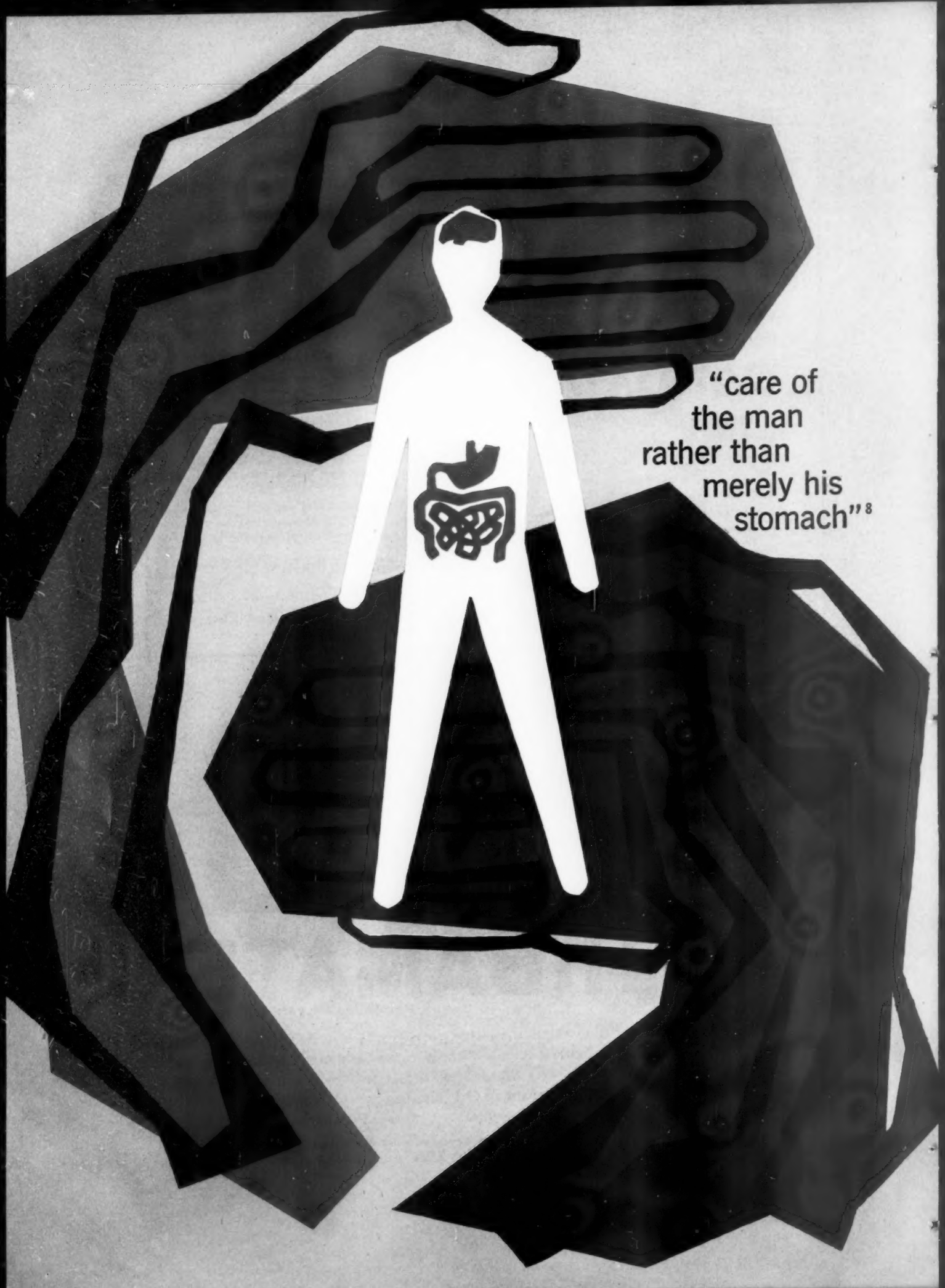
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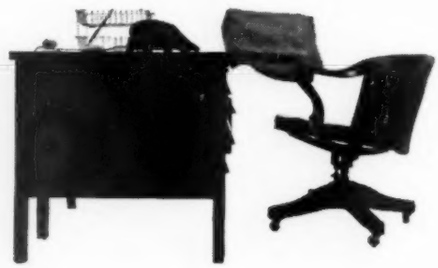

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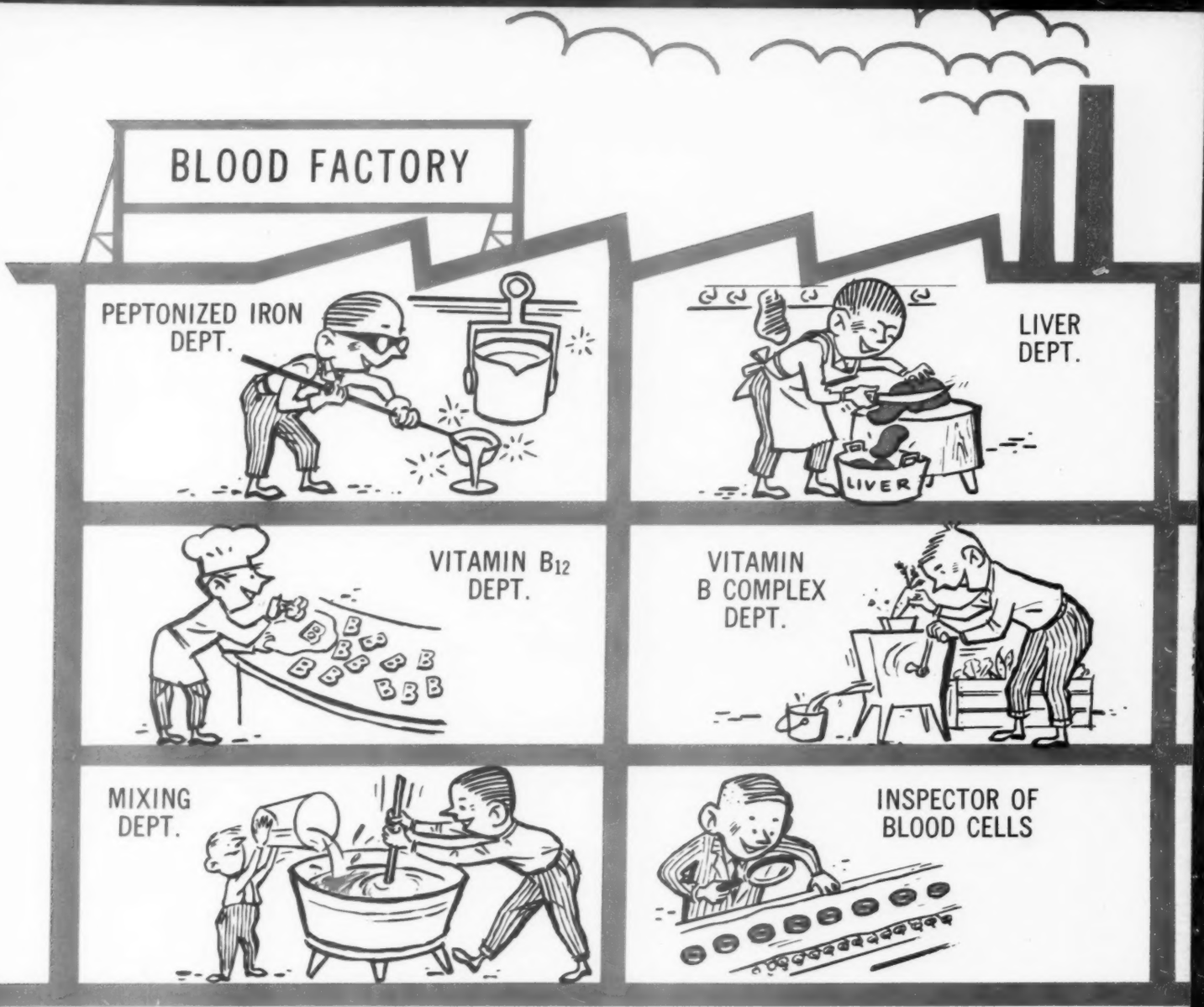

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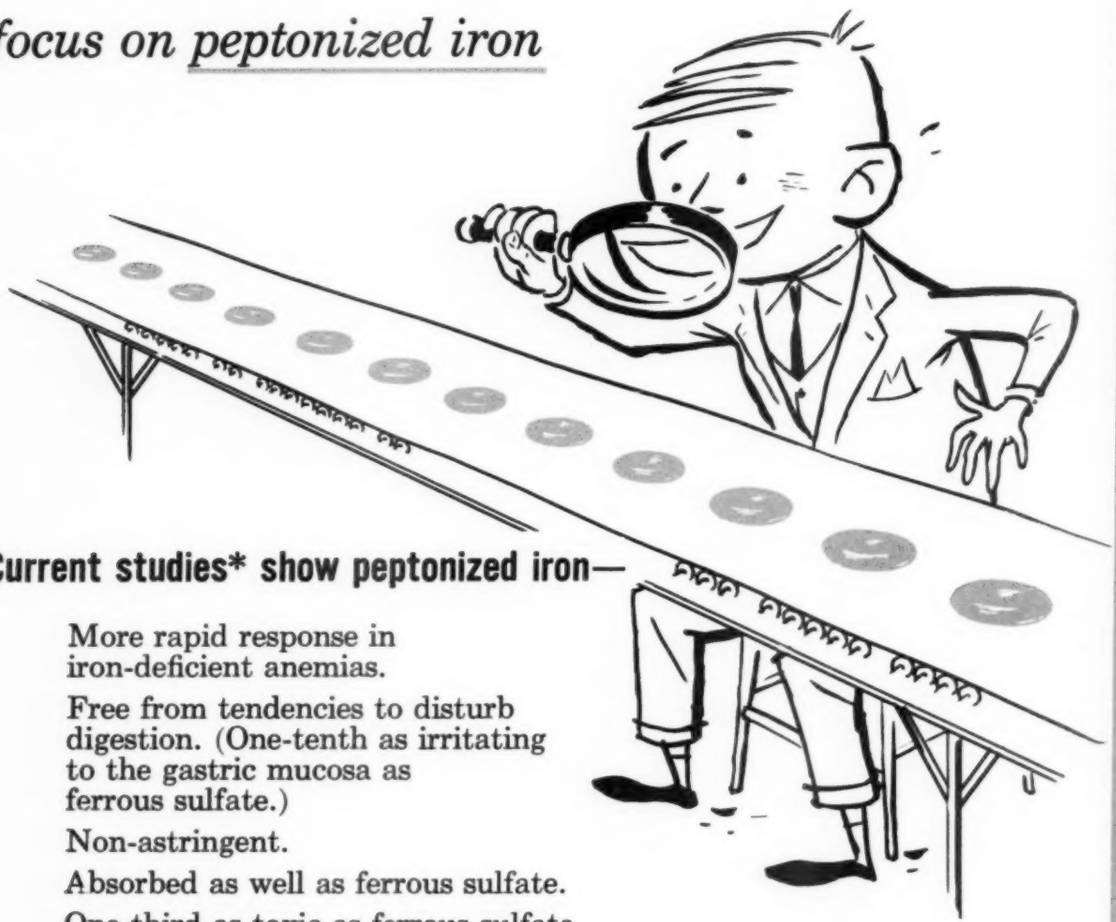

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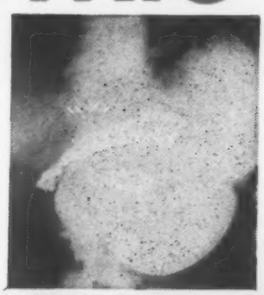

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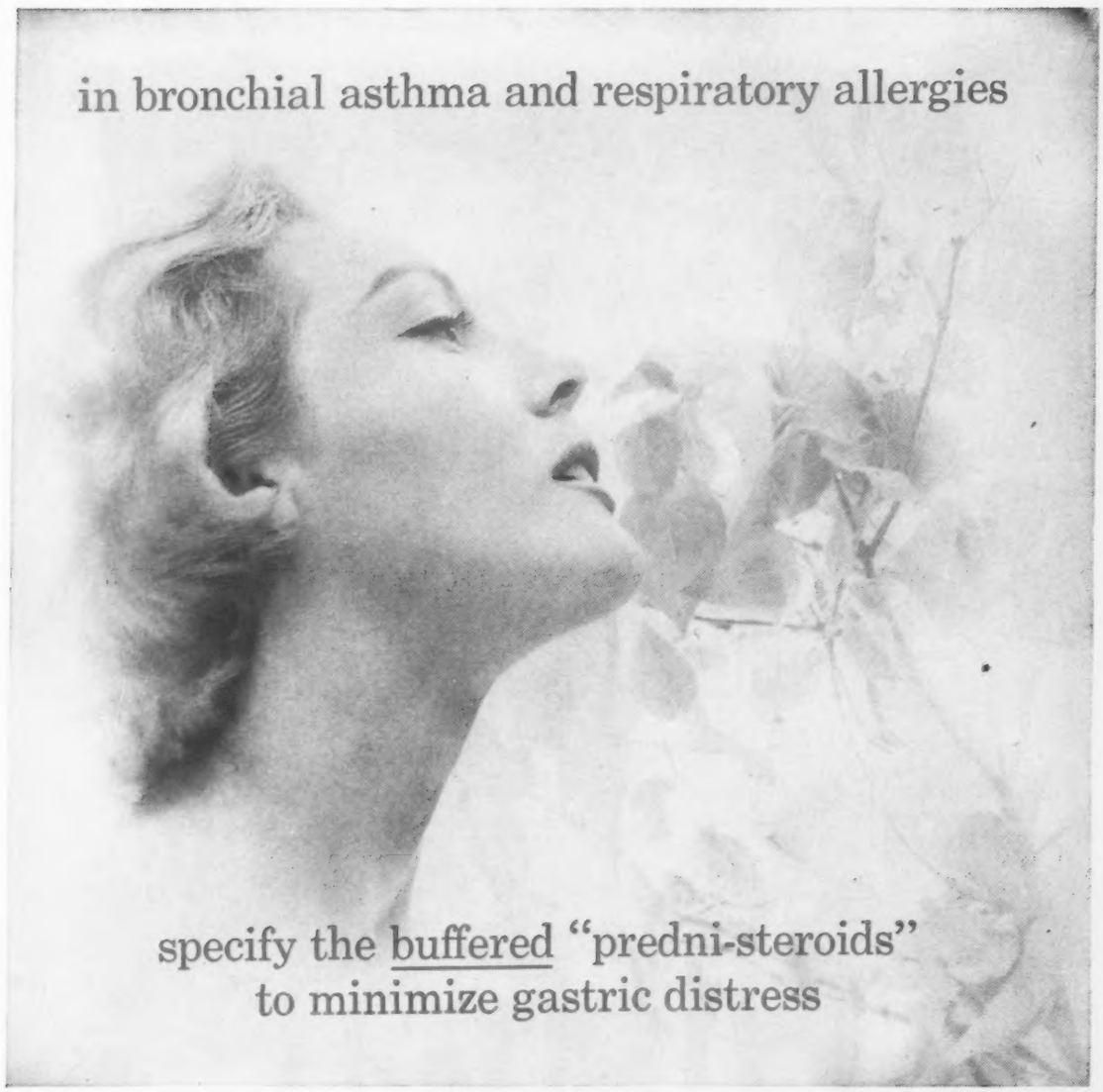

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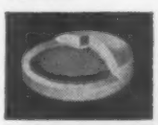

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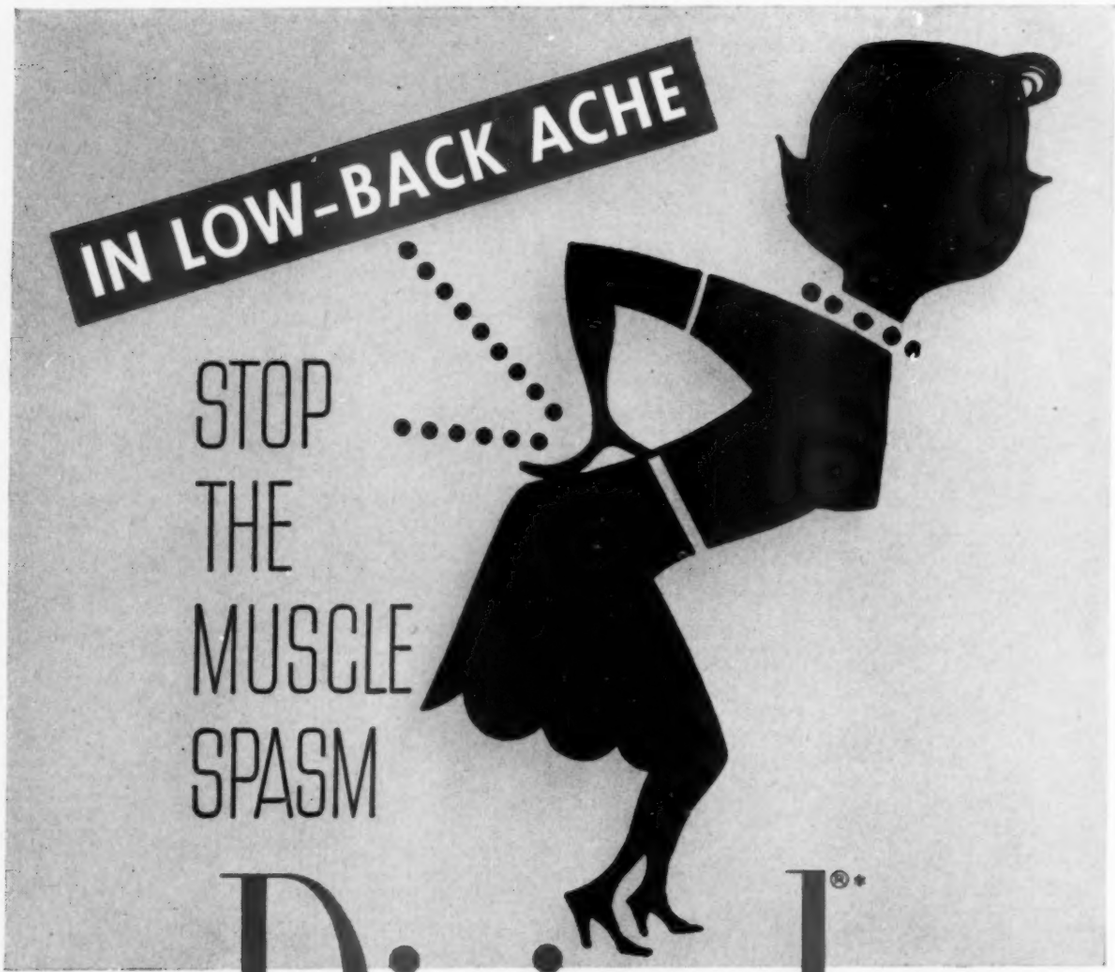

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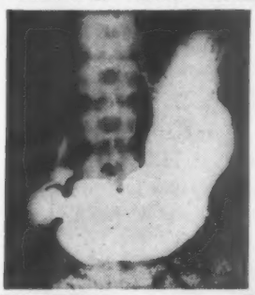

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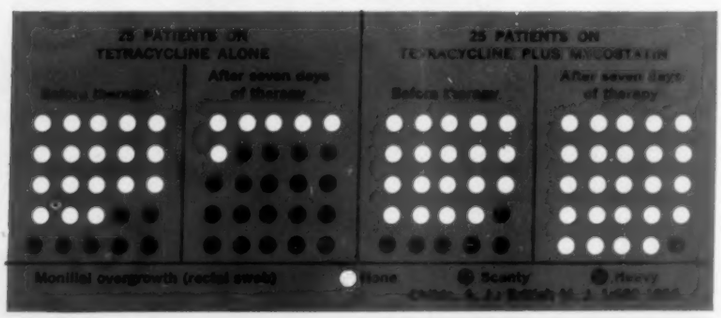

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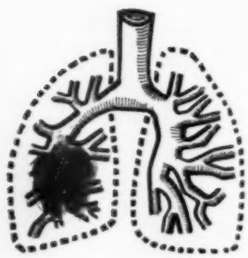

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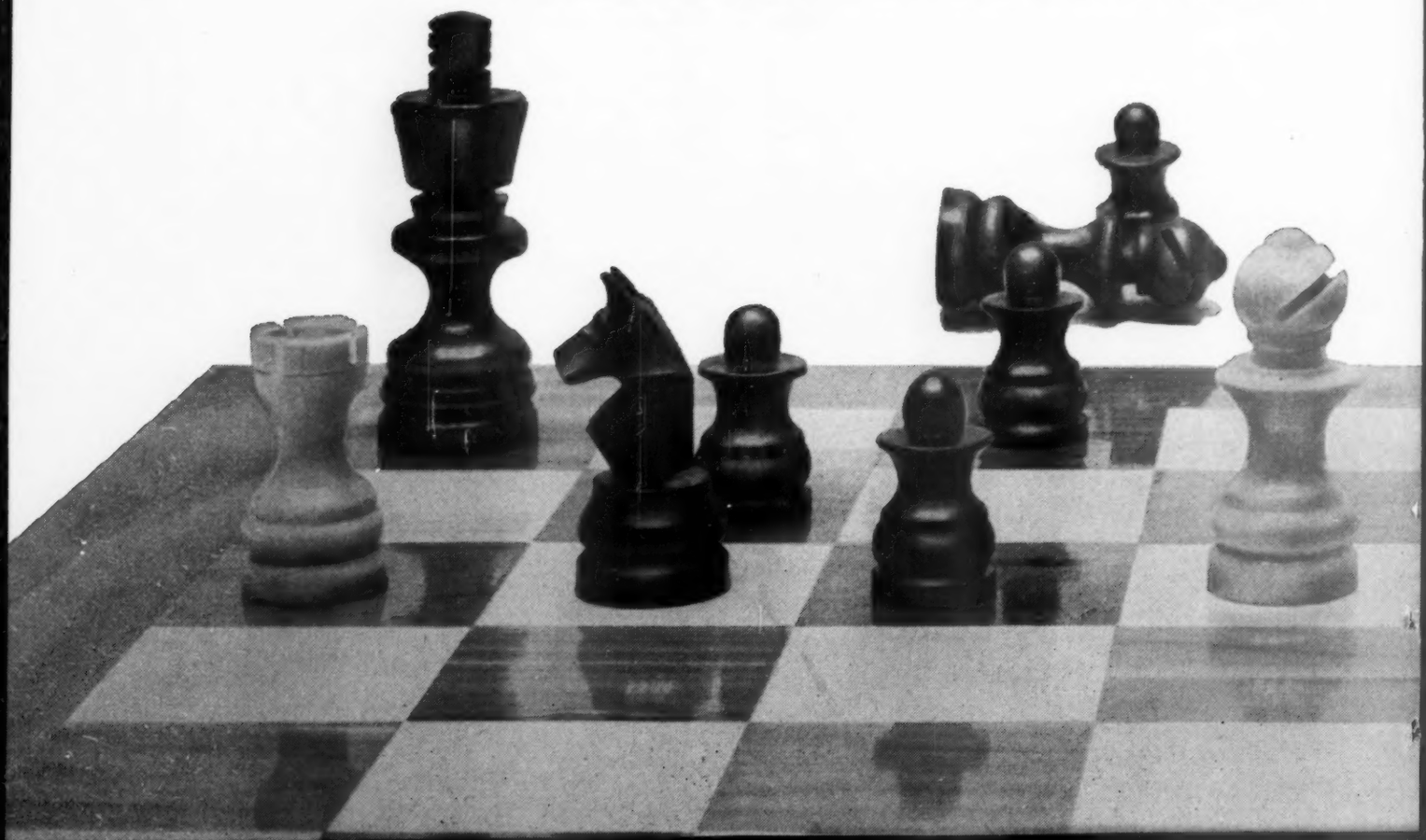

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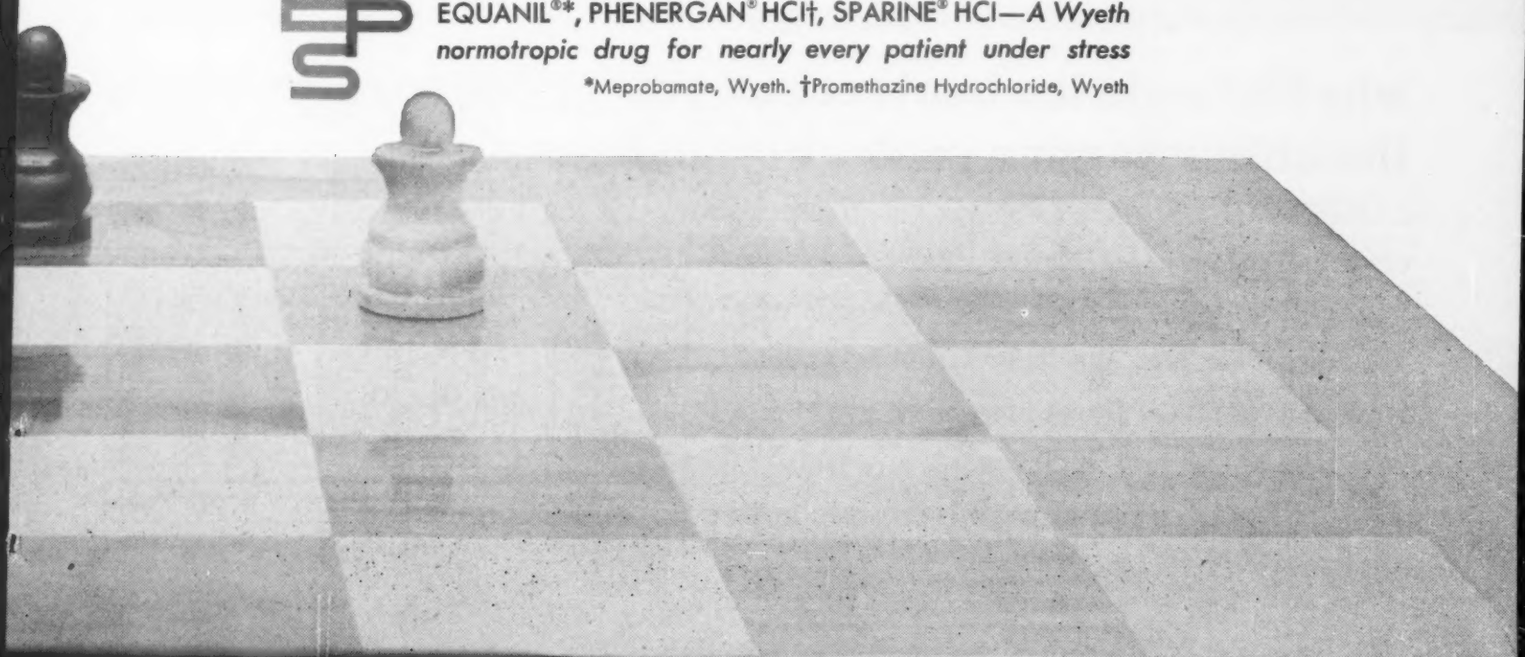

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Urticaria and angioneurotic edema	3	1	1	1		Dizzy (1)
Allergic dermatitis	2		1	1		Slight Drowsiness (2)
Bronchial asthma	1		1			
Pruritus	1		1			
Total	37	15	13	7	2	Drowsiness (5) Dizzy (1) 16.2%

From the preliminary **Dimetane Extentabs** studies of three investigators. Further clinical investigations will be reported as completed.

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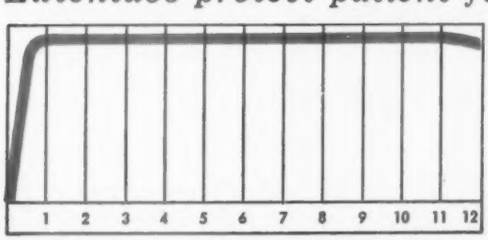

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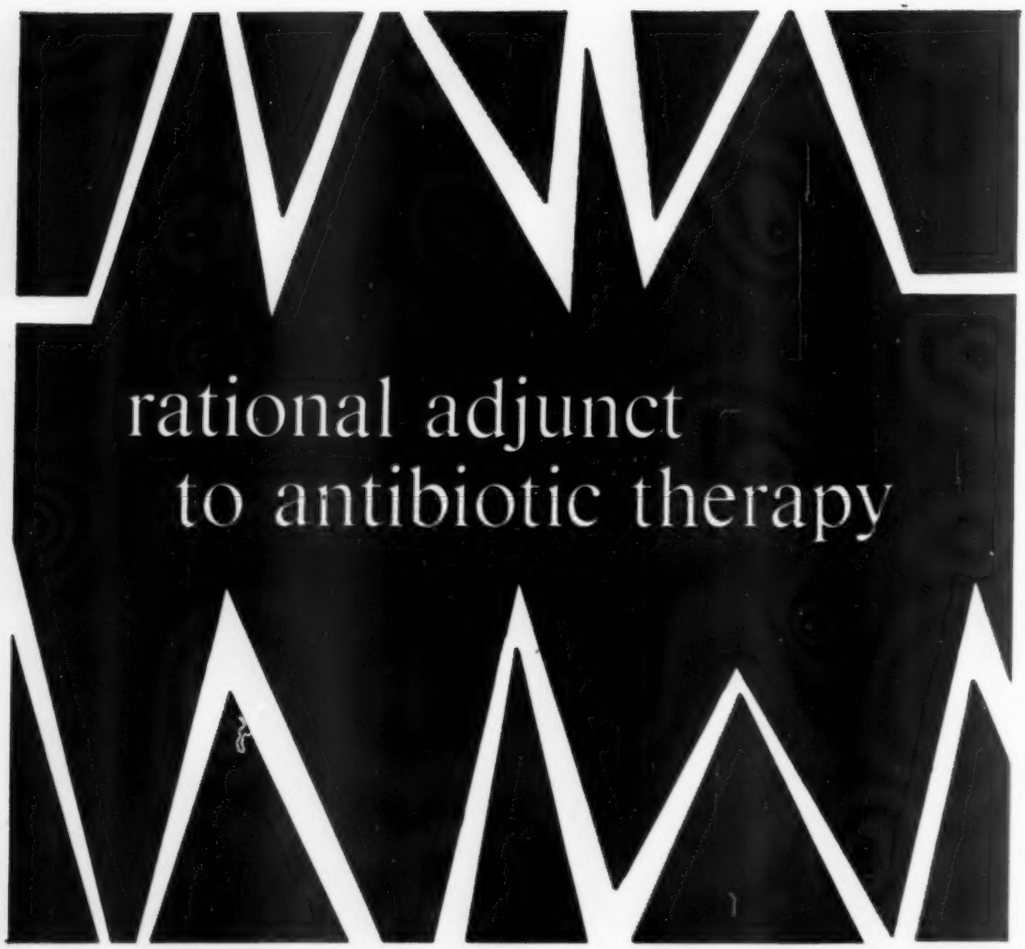

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1. Pratt, R.: *Geriatrics* 11:341 (June) 1957. 2. Pulaski, E.J.: *Antibiotics Annual* 1953-1954, Proceedings of the Symposium on Antibiotics Sponsored By U. S. Department of Health, Education and Welfare, Food and Drug Administration, Division of Antibiotics, 1953, Medical Encyclopedia, Inc., New York, p. 227.

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1. Lombroso, C. T., Davidson Jr., D. T., and Grossi-Bianchi, M. L.: Further Evaluation of Acetazolamide (DIAMOX) in Treatment of Epilepsy, J.A.M.A. *160* 268-272, 1956.

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Ford, R.V., and Moyer, J.H.: Rauwolfia Toxicity in the Treatment of Hypertension: Some Observations on Comparative Toxicity of Reserpine, a Single Alkaloid, and Alseroxylon, a Compound Containing Multiple Alkaloids, Postgrad. Med., January, 1958.

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When more potent drugs are needed, prescribe

Rauwiloid® + Veriloid®
alseroxylon 1 mg. and alkavervir 3 mg.
for moderate to severe hypertension.
Initial dose 1 tablet t.i.d., p.c.

Rauwiloid® + Hexamethonium
alseroxylon 1 mg. and hexamethonium chloride dihydrate 250 mg.
in severe, otherwise intractable hypertension.
Initial dose ½ tablet q.i.d.

Both combinations in convenient single-tablet form.

advanced therapy for advancing hypertension

Apresoline®

hydrochloride

(hydralazine hydrochloride CIBA)

- to lower blood pressure
- to increase renal blood flow
- to decrease cerebral vascular tone

SUPPLIED: Ampuls, 1 ml., 20 mg. per ml.

Tablets, 10 mg. (yellow, double-scored),
25 mg. (blue, coated),
50 mg. (pink, coated);
bottles of 100, 500 and 1000.

Tablets, 100 mg. (orange, coated);
bottles of 100 and 1000.

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